

**The Effect of Temperature on Lignin Degradation  
in Municipal Solid Waste**

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## (ABSTRACT)

Paper and paperboard are the major constituents found in US landfills. Typically paper consists of 79% to 98% of lignocellulose which is considered to be the most abundant source of natural carbon on earth. Lignocellulose decomposition depends on the association of biodegradable cellulose and hemicellulose with lignin. Lignin is a recalcitrant material which hinders cellulose degradation in conventional landfills. Because of this property of lignin cellulose to lignin ratio (C/L) is a common landfill stabilization parameter. Refuse degradation in landfills is a microbiological process and is highly dependent on temperature, moisture, and pH. Bioreactor landfills are designed to enhance biodegradation of refuse by providing favorable conditions for microorganisms. Effect of elevated temperature and moisture on possibility of lignin degradation is studied in this work. Synthetic and newspaper lignin were preheated and then inoculated with anaerobically digested sludge. Newspaper in distilled water exposed to 95°C for 48 hours released 8 times more of solubilized lignin than non preheated newspaper. Moreover lignin monomers were detected as a result of 95°C pretreatment indicating the positive effect of high temperature on the providing lignin in more bioavailable form for microbes. Digested sludge inocula was found to be capable of lignin monomers degradation as well as low but significant mineralization of synthetic lignin with approximately 6% of carbon originated from lignin mineralized into methane and carbon dioxide. An exponentially increasing trend for lignin monomers solubilization as a function of temperature was observed for three types of substrate, synthetic lignin, cardboard, and newspaper with the highest rate of solubilization for newspaper. Results of this study suggest that some lignin degradation can occur at conditions typical for bioreactor landfills.

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# CHAPTER 1: LITERATURE REVIEW

## 1.1 Paper in MSW: generation and utilization

The refuse generated by human activities is classified by the US EPA into the following categories: households and commercial, industrial, hazardous, and construction wastes. Household and commercial types are also known as municipal solid wastes (MSW). It includes food scraps, package wrapping, grass clippings, furniture, and electronic devices. In terms of the materials it consists of paper, plastic, wood products, metals and food. The US EPA continuously monitors the rates of generation of wastes as well as its utilization paths. Paper is a number one among the MSW constituents generated in the USA (Figure 1.1). For some other countries such as Germany, Spain, UK, and Australia, paper does not dominate, but still is one of the major contributors to the MSW (Barlaz *et al.*, 2006).



Figure 1.1: The percentage by which different materials contribute to the municipal solid waste stream (US EPA: 2003).

Since 1980, the total annual MSW generation in the USA increased by 50 percent to its 2003 level of 236 million tons (US EPA: 2003). The majority of MSW is disposed at landfills. However, in recent years the European Union (EU) has begun implementing the directive which prohibits disposal of degradable organic matter. To divert some of the

refuse from disposal – recycling, including composting, is one of the alternatives which reduces the MSW volume. Total materials recovery for recycling and composting has reached 30 percent since 2000 in the USA (US EPA: 2003). Although, paper and paperboard materials are recycled only up to 48.1 percent (Figure 1.2), which is less than percent recovery for auto batteries, steel cans and yard trimmings, it is number one in recycling in terms of the weight recovered – about 40.0 million tons in 2003 (US EPA: 2003).

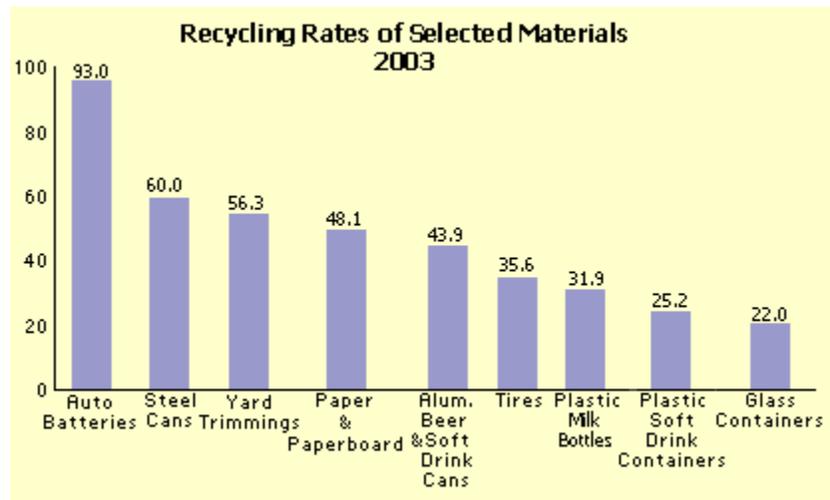


Figure 1.2: The recycling rates of selected materials in USA at 2003 (US EPA: 2003).

Another alternative for refuse volume reduction is combustion. Because of increasing concerns about air emissions, it is not popular in the USA and only about 14 percent of the generated refuse is burned at combustion facilities. In contrast, in Japan approximately 78 percent of MSW is combusted (Barlaz *et. al.*, 2006).

### 1.2 Landfills

Nowadays landfills are well-engineered facilities designed and operated in a way to protect the environment from contaminants which may be present in the solid waste

stream. For instance, hazardous wastes are not allowed at sanitary or conventional landfills for MSW. However, leachate formed from the liquid contained in waste and atmospheric precipitation are considered to be very toxic if infiltrates to the aquifer and soil. Despite an increasing interest in alternative approaches to treat MSW, about 55.4 percent of the refuse ends up disposed at landfills in USA (US EPA: 2003). Interestingly, the item most frequently encountered in landfills is paper and paperboard. Paper materials account for more than 40 percent of a US landfills by mass and volume with about 13 percent contributed by the newspaper alone (Rathje and Murphy, 1992; Micales and Skog *et. al.*, 1997).

***Phases of microbial activities in landfills***

Decomposition of biodegradable organic matter in landfills is a microbiological process. Four phases of different microbial activities with the utilization of certain substrates were characterized: aerobic phase, anaerobic acid phase, accelerated methane production phase, and decelerated methane production phase (Barlaz, Schaefer and Ham *et. al.*, 1989; Micales and Skog *et. al.*, 1997; Barlaz *et. al.*, 2006). These phases can be described with the schematic adopted from El-Fadel (*et. al.*, 1997) which highlights major inter-dependent steps occurring at landfill (Figure 1.3).

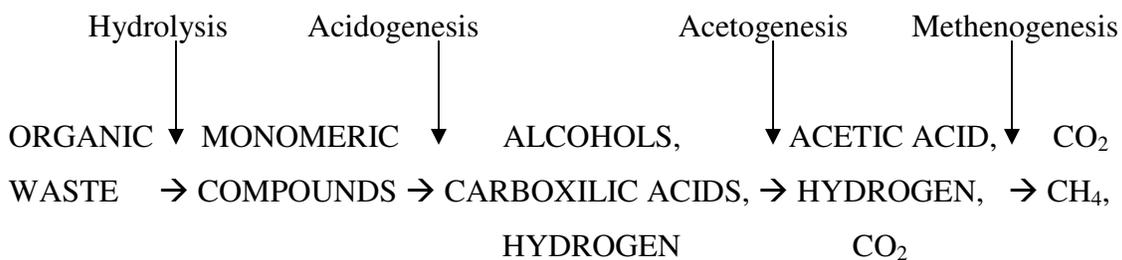
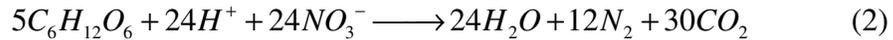
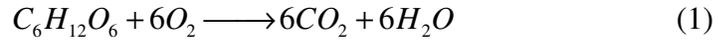


Figure 1.3: Major steps of the anaerobic decomposition of refuse.

During the initial aerobic phase the depletion of the oxygen and nitrate in the refuse occurs with the large amount of the CO<sub>2</sub> generated at the first phase. Reaction equations (1) and (2) describe this process in general (Barlaz, Schaefer and Ham *et. al.*, 1989). Then

the environment becomes oxygen-limiting, although some atmospheric oxygen can still infiltrate through the daily cover and migrate in the areas of poorly compacted wastes.



In the second stage a diverse population of hydrolytic and fermentative bacteria hydrolyzes polymers such as lignocellulose, proteins, lipids into soluble intermediates: soluble sugars, amino acids, long-chain carboxylic acids, and further to short-chain carboxylic acids (eg.: acetic, propionic, butyric, valeric, capronic acids), alcohol, hydrogen, and carbon dioxide. This stage is also characterized by the accumulation of carboxylic acids, which together with hydrogen causes the drop of pH from 7.5 to about 5.7, and little solids decomposition. However, at the end of this phase, more acetate is available and bacteria start to utilize it to generate gas. This leads to an increase the pH (Barlaz, Schaefer and Ham *et. al.*, 1989). At the same time the pH starts to recover to the level of a neutral due to the release of metallic cations from the reaction of the acids on the metallic components of the refuse (Micales and Skog *et. al.*, 1997).

The third phase is characterized by a decrease in the accumulation of carboxylic acids and an intensification of methane production. Significant biodegradation of the cellulose and hemicellulose occurs and the methanogenic bacteria reach their maximum level (Barlaz *et. al.*, 2006). The processes occurring at this stage can be described by the “corporate” activity or syntrophy of acetogens and methanogens. Syntrophy is a simultaneous substrate degradation by two or more different microorganisms, that neither can degrade separately (Madigan, 2000). Acetogens (hydrogen-producing) continue to oxidize remaining short-chain carboxylic acids to acetate, hydrogen and carbon dioxide. Although these reactions are thermodynamically unfavorable in the presence of hydrogen and require the removal of hydrogen as it is produced, methanogens utilize hydrogen to produce methane and to provide conditions for acetogens, equations (3) and (4). Methanogens are very sensitive to several parameters such as temperature, pH, toxins, and if no methanogenic population is established at some part of landfill it means that

system is not balanced and little biodegradation can be expected. Hence, methane generation is a rate limiting factor of decomposition (Micales and Skog *et. al.*, 1997), and serves as an indicator of the organic waste biodegradation.



In the fourth phase carboxylic acids become depleted and the rate of the CH<sub>4</sub> generation depends on the rate of the lignocellulose hydrolysis providing the maximum rate of solids decomposition (Barlaz, Schaefer and Ham *et. al.*, 1989). As a result of this phase refuse become a stabilized soil-like material unable to be further degraded.

#### ***Factors influencing the degree of biodegradation***

Landfills are very heterogeneous microenvironments and different areas may undergo different phases of degradation. It is a common practice to find areas of undegraded wastes close to well degraded materials. There are several factors which influence the biological activity in landfills such as moisture, pH, temperature, bioavailability, composition of waste, design and operation of the landfill facility.

Laboratory studies show that if pH drops to about 5 and no pH adjustment is applied, a strong inhibition of methanogens occurs and accumulation of carboxylic acids leads to the further deterioration of refuse hydrolysis and acidogenesis (Vavilin *et. al.*, 2003).

Anaerobic degradation at landfills is a microbial process and is highly affected by temperature. The methane generation rate is one of the parameters to estimate the efficiency of the landfill operation. Laboratory study of MSW decomposition proved that methanogenic bacteria produce a significantly greater amount of gas when operated at thermophilic temperature range (50°-60°C) (Garba *et. al.*, 1996).

Recent studies have concluded that moisture of the buried refuse is the most critical factor enhancing biodegradation (Kinman *et. al.*, 1987; Gurijala and Suflita *et. al.*, 1993; San and Onay *et. al.*, 2001; Kim and Pohland *et. al.*, 2003). However, current regulations in USA restrict the elevated moisture at landfills because rainwater and moisture from the refuse form large quantities of leachate which is hazardous if it infiltrates to groundwater, soil or surface watershed and requires *ex-situ* treatment (Micales and Skog *et. al.*, 1997).

### ***USA Regulations***

US congress enacted the Waste Disposal Act of 1965 because of the growing rates of wastes generation as well as the necessity to protect human health and environment from potential hazard. That was the first act specifically regulating solid waste disposal methods and practice in USA. Then, as public and government became more concerned with waste handling, the 1976 Resource Conservation and Recovery Act (RCRA) was enacted. “The primary goals of RCRA are:

- To protect human health and the environment from the hazards posed by waste disposal
- To serve energy and natural resources through waste recycling and recovery
- To reduce or eliminate, as expeditiously as possible, the amount of waste generated, including hazardous wastes
- To ensure that wastes are managed in a manner that is protective of human health and the environment”

Three programs within RCRA were promulgated to achieve these goals. One of the programs is devoted to non hazardous solid wastes and is named Subtitle D. According to the act, state and local governments regulate waste handling and disposal as well as the operation of landfills and incineration facilities. However, EPA establishes rules of how these facilities should be designed and operated to provide safety for people and environment. For instance, EPA requires disposal facilities to install safety measures to control explosive gas emissions from the degrading wastes and to prevent contamination

of soil and ground water from the infiltration of toxic leachate (USEPA: RCRA SUBTITLE D, 2006).

According to USEPA there are four general types of landfills. The EPA specifies design and operation criteria for each type. These are:

- Municipal solid waste (conventional/sanitary);
- Bioreactor;
- Construction and demolition debris;
- Industrial wastes.

### *1.3 Bioreactor landfills*

Bioreactor landfills originate from studies of the implementation of leachate recirculation for faster refuse stabilization conducted by Pohland as early as at 1975 (Kim and Pohland *et. al.*, 2003). It was proved that sequential leachate recirculation allows reducing of the toxicity of the leachate and, hence, a decrease in the capital costs of its *ex-situ* treatment. Moreover, it introduces additional moisture which enhances the microbiological processes of waste decomposition (Pohland *et. al.*, 1994; Pohland *et. al.*, 1995; Reinhart and Townsend, 1998; Vavilin *et. al.*, 2002; Warith *et. al.*, 2002; Barlaz *et. al.*, 2006). As more and more studies were conducted, the idea of landfill as bioreactor became more and more popular. Recently a number of bioreactor landfill pilot projects have been run in USA with the approval of US EPA. The EPA selected facilities and granted them regulatory flexibility that allows them to recirculate leachate and add moisture to landfills constructed with an alternative liner system to prevent leachate infiltration from the landfill. The amount of leachate formed at landfills usually is not enough to sustain optimal conditions for an effective landfill operation as a bioreactor. The character of other liquids and semi-liquids to apply depends on the regulatory approvals and can be water, stormwater, wastewater treatment sludge, wastewater (Reinhart and Townsend, 1998; Warith *et. al.*, 2002). Moreover, liquid movement through layers of refuse

distributes inocula and nutrients more evenly and dilutes potential toxins (Vavilin *et. al.*, 2002). Bioreactor landfills have real advantages to conventional “dry tomb” landfills and these advantages bring greater responsibilities. For instance, intense LFG generation from bioreactors requires collection and utilization: better quality of LFG from landfills allows using it as energy source. Liners should protect soil and ground water under landfill from seeping of bulk liquids applied to maintain desired moisture. Other design modifications can be required to provide the protection for human health and environment. Operators have to be trained to manage the increased complexity of the facility (Barlaz and Reinhart *et. al.*, 2004).

### ***Types of bioreactor landfills***

Depending on the target microbial population, aerobes or anaerobes, and waste composition bioreactors have different design and operational features. According to US EPA “there are three different general types of bioreactor landfill configuration:

- *Aerobic*: In an aerobic bioreactor landfill, leachate is removed from the bottom layer, piped to liquids storage tanks, and re-circulated into the landfill in a controlled manner. Air is injected into the waste mass, using vertical or horizontal wells, to promote aerobic activity and accelerate waste stabilization.
- *Anaerobic*: In an anaerobic bioreactor landfill, moisture is added to the waste mass in the form of re-circulated leachate and other sources to obtain optimal moisture levels. Biodegradation occurs in the absence of oxygen (anaerobically) and produces landfill gas. Landfill gas, primarily methane, can be captured to minimize greenhouse gas emissions and for energy projects.
- *Hybrid (aerobic-anaerobic)*: The hybrid bioreactor landfill accelerates waste degradation by employing a sequential aerobic-anaerobic treatment to rapidly degrade organics in the upper sections of the landfill and collect gas from lower sections. Operation as a hybrid results in the earlier onset of methanogenesis compared to aerobic landfills.”

### ***The difference between conventional and bioreactor landfills***

For microbiological systems such as landfills, moisture is the key factor influencing rates of metabolism for organisms. Conventional landfills usually have about 20 percent moisture whereas bioreactors require at least 40 percent with optimal range of 40 to 60 percent to sustain enhanced biodegradation of refuse (Gurijala *et. al.*, 1997, Bagchi, 2004). As was mentioned previously, leachate recirculation and addition of other bulk liquids provides sufficient conditions to increase microbial activity, which in turn significantly increases mineralization of waste into LFG. The rate of gas generation also decreases relatively soon (measured in years) as opposed to conventional landfills which typically continue to emit LFG for decades after closure (Figure 1.4) (Micales and Skog *et. al.*, 1997; Bagchi, 2004).

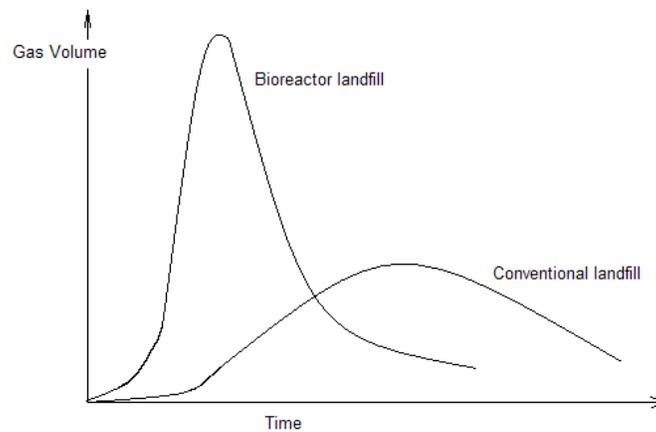


Figure 1.4: Comparison of idealistic gas generation rates between conventional (sanitary) and bioreactor landfills (adopted from Bagchi, 2004).

At bioreactor landfills enhanced degradation of readily and moderately bioavailable organic waste occurs so it takes about 5 to 8 years of operation to stabilize MSW. Here stabilization means that such parameters as composition and rates of LFG generation as well as leachate constituent concentrations from the landfill do not pose any risk to the environment under any circumstances. Even in case of partial failures of containment system after the closure of bioreactor (Warith *et. al.*, 2002). When the landfill is filled,

postclosure care activities for sanitary landfill typically last for 30–50 years (Tchobanoglous, 2002).

Leachate recirculation in bioreactor landfills is also advantageous for the settlement of buried waste. As a result of laboratory and pilot studies higher rates of compaction compared to conventional landfill were measured. Since wastes undergo different stages of biodegradation, the highest settlement rate occurs when solid-to-gas conversion takes place and LFG is intensively generated (Warith *et. al.*, 2002). For instance, the pilot-scale study of Sonoma County Landfill, CA, showed that leachate recirculated cells had 20 percent settlement of refuse initial depth, and dry cells settled for less than 8 percent. Another study at the Mountain View Landfill, CA, showed approximately 13–15 percent compaction of a wet cell compared to about 8–12 percent for the control “dry” cell in four years of operation. Thus, well compacted waste increases landfill capacity before closure. It allows disposing of more MSW within one unit. It also reduces maintenance costs for final cover: damage of which is less probable compared to sanitary landfills. However, enhanced compaction, if not predicted and foreseen, may cause damage of leachate distribution and LFG collection pipe systems (Reinhart and Townsend, 1998).

#### *1.4 Methane generation*

MSW buried in landfills contain a vast amount of organic carbon which is partially mineralized into methane and carbon dioxide and released to the atmosphere. There are many studies that have investigated the rate of methane generation from different types of landfills and different biodegradable fractions of MSW (Kinman *et. al.*, 1987; Gurijala and Suflita *et. al.*, 1993; Micales and Skog *et. al.*, 1997; Kim *et. al.*, 1997; Warith *et. al.*, 2002; Barlaz *et. al.*, 2006). The methane generation rate attracts the attention of researchers because methane can be used as an energy source. Some big sanitary landfills as well as bioreactors may produce methane sufficient to provide energy for the landfill operation or to be purified and sold. Conventional landfills generate methane which can be used only for 5 to 20 years. The rest of the time it is collected and burned on site or

released to the atmosphere. Old landfills continue to emit low concentrations of LFG for decades (approximately 8 to 40 years) after closure (Micales and Skog *et. al.*, 1997). Moreover, methane is a greenhouse gas which is about twenty times more potent than carbon dioxide at blocking the heat loss from the earth (Barlaz *et. al.*, 2006). To improve this situation, bioreactor technologies make an attempt to create conditions which provide mineralization of almost all the biodegradable refuse at the highest rate and concentration occurring in years of operation (Figure I.4). Laboratory studies showed methane production can reach about 60 percent of LFG at a rate of 126 L/year-kg (dry wt) from landfill simulation reactors with neutralized leachate recirculation to maintain 73 percent of moisture in refuse (Barlaz *et. al.*, 1989).

In literature several models have been proposed to predict methane generation and emission from the landfill. These models are generally zero-order, first-order, multiple-phase, and second order. Zero order models usually describe a linear dependence of the LFG rate from refuse mass, and such important factors as moisture and density/compaction of waste are assumed to make no difference (Micales and Skog *et. al.*, 1997). For example, EPA LandGEM model (2005) for gas generation from landfill uses the first order kinetic equation (5).

$$Q_{CH_4} = \sum_{i=1}^n \sum_{j=0.1}^1 kL_o \left( \frac{M_i}{10} \right) e^{-kt_{i,j}} \quad (5)$$

where  $Q$  = annual methane generation rate for specific year  $t$  ( $m^3CH_4$  / year);

$i, j = 1$  and  $0.1$  year time increment relatively;

$n$  = year of the calculation minus initial year of waste acceptance;

$M$  = mass of waste buried annually (ton / year);

$L_o$  = methane generation potential ( $m^3CH_4$  / Mg of waste);

$t$  = time after initial waste placement (year);

$k$  = first order decay rate constant ( $year^{-1}$ );

$t_{ij}$  = age of the  $j$ -th section of waste mass  $M_i$  in the  $i$ -th year.

Multi-phase models usually include several first-order models for different parameters influencing LFG generation rate. The Second order model was designed to take into account the complex chemical and microbiological processes affecting methanogenesis. However, after comparison of predicted values from different types of models, it was concluded that multi-phase, first- and second-order models were similar with multi-phase being slightly more accurate in describing LFG generation rates. Another important factor which is usually not considered by these models is that not all the methane produced reaches the atmosphere. Approximately 10% of generated methane is oxidized by the soil-inhabiting bacteria present in landfill's cover soil. It should be taking into account for more accurate prediction of impact of LFG to the environment (Micales and Skog *et. al.*, 1997).

### *1.5 Lignocellulose anaerobic degradation*

The composition of MSW, as it was previously described, can be very diverse. Although the composition varies from place to place and depends on season, community activity (amount of food places, households and businesses), climate, etc., MSW consists primary of lignocellulose or lignin carbohydrate complexes (LCCs). LCCs is a general term for lignin, cellulose and hemicellulose – major components of arborescent plant tissue. The percentage of LCCs in fresh MSW reported in literature by different investigators and summarized by Barlaz (*et. al.*, 2006) represents either residential refuse as collected or as delivered to landfill, in this case the commercial, demolition and construction waste is included (Table 1.1).

Nowadays researches consider lignocellulose to be the most abundant natural carbon source on earth. Cellulose is a water insoluble unbranched polymer containing several thousand of D-glucose units linked by  $\beta$ -1,4-glycosidic bonds. Hemicellulose is a poorly defined group of water insoluble poly saccharides such as L-arabinose, uronic acid, and hexoses (D-mannose, D-galactose and D-glucose). Lignin, in turns, is a highly branched polymer consisting of aromatic units randomly joined by carbon-carbon and ether bonds.

It is at best very slowly degradable and considered to be recalcitrant. The overall schematic of lignicellulose structure as a component of wood was proposed by P. J. Colberg (1988) (Figure 1.5). Therefore the information about chemical composition is important for better insight of the waste biodegradation in landfills, especially bioreactors.

Different constituents of waste have different percentages of cellulose, hemicellulose and lignin, and it affects the rate of decomposition. Paper composition is of particular interest because of both its wooden-origin nature and relatively big fraction of MSW. Barlaz (*et. al.*, 2006) summarized the distribution of LCCs components for major waste materials (Table 1.2). Hence, the association of cellulose and hemicellulose to lignin makes a difference for the biodegradation. Paper-cellulose tends to degrade faster than wood-cellulose because it was processed and fibers were partially destroyed (Micales and Skog *et. al.*, 1997). Similarly, newspaper is degraded slower than office paper because it contains “all of the lignin of wood pulp” and office paper has the least amount of lignin left (Barlaz *et. al.*, 1997). Recent studies showed that cellulose and hemicellulose are considered to be the main carbon sources in landfills, and their degradation provides 90 percent of the total methane produced by the refuse (Rodriguez *et. al.*, 2005).

Table 1.1: Organic composition of residential refuse expressed as percent of dry weight (adopted from Barlaz *et.al.*, 2006).

Source <sup>a</sup>	A	B	C	D	E	F	G	H
Cellulose, C	42.4	25.6	63.4	51.2	28.8	38.5	48.2	43.9
Hemicellulose, H	no data	6.6	no data	11.9	9.0	8.7	10.6	10.0
Lignin, L	10.9	7.2	15.7	15.2	23.1	28.0	14.5	25.1
(C+H)/L	3.89	4.5	4.04	4.15	1.64	1.68	4.06	2.15
Volatile solids	no data	59.6	no data	78.6	75.2	no data	71.4	no data
Source	landfill	landfill	landfill	residential refuse				

<sup>a</sup> For more details about sources see Barlaz (*et.al.*, 2006).

Table 1.2: Chemical composition of major MSW materials (adopted from Barlaz *et.al.*, 2006).

Source <sup>a</sup>	Newsprint		Office paper		Cardboard
	1	2	1	2	2
Cellulose	48.3	48.5	64.7	87.4	57.3
Hemicellulose	18.1	9	13.0	8.4	9.9
Lignin	22.1	23.9	0.93	2.3	20.8
Volatile solids	98.0	98.5	88.4	98.6	92.2

<sup>a</sup> For more details about sources see Barlaz (*et.al.*, 2006).

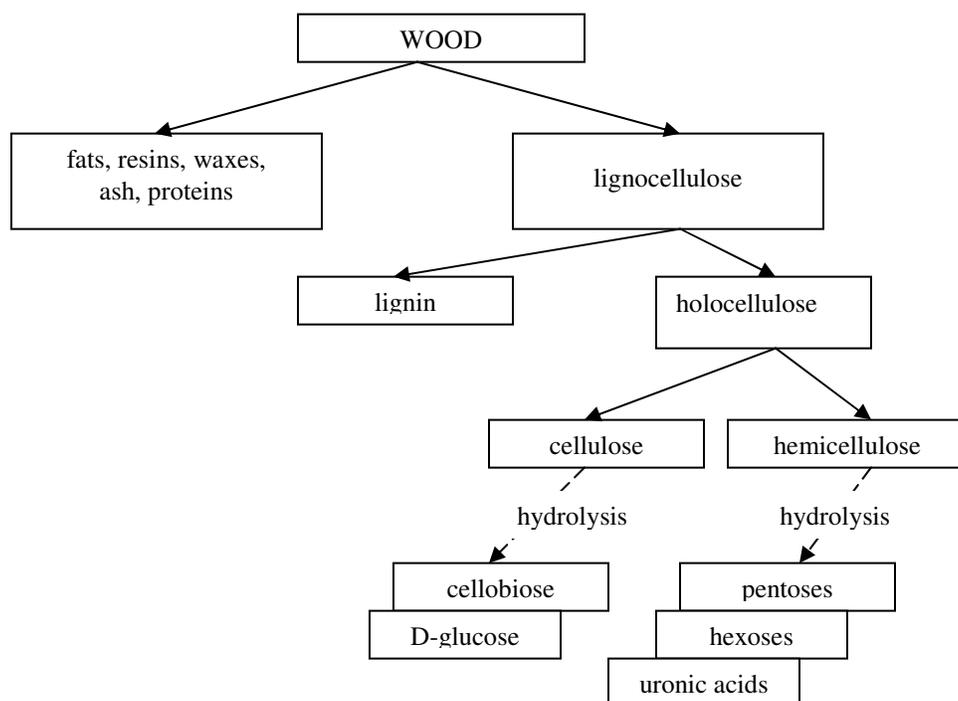


Figure 1.5: Wood composition, including cellulose, hemicellulose with their hydrolysis products (adopted from Colberg, 1988).

### *1.6 Lignin anaerobic degradation pathways*

One of the major sources of anaerobic recalcitrance of the refuse is the poor uptake of non-hydrolysable and slow dissolving polymers by landfill microbial consortia. Such polymers, lignin, plastic, and humic compounds, highly affect bioavailability of organic waste (Field *et. al.*, 2002). Thus, lignin is considered to be a recalcitrant compound, which limits cellulose and hemicellulose degradation.

Laboratory studies of anaerobic lignin degradation by acclimated enriched cultures use either synthetic model compounds, which contain intermonomer linkages and functional groups similar to natural lignin, or lignin degradation intermediates (oligomers and/or

lignin monomers). As a result of Young and Fraser studies (*et. al.*, 1987) lignin degradation pathways were proposed (Figure 1.6).

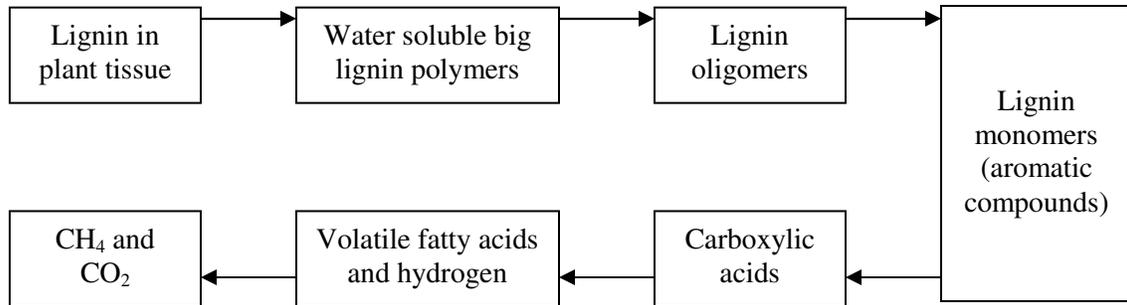


Figure 1.6: Generalized schematic of lignin anaerobic biodegradation pathway.

The investigation of lignin anaerobic degradation potential conducted by Young and Fraser (*et. al.*, 1987) can be summarized as the following:

- Acclimated mixed enrichments are not only capable of partial mineralization, but also solubilize lignin;
- Complex lignin polymers can undergo a small but significant partial biodegradation by some acclimated mixed cultures;
- Lignin oligomers such as dimers and trimers are depolymerized (Figure 1.7) and metabolized under laboratory conditions;
- Lignin monomers (eg.: vanillic, ferulic, syringic, benzoic acids, and catechol) can be readily metabolized and mineralized anaerobically.

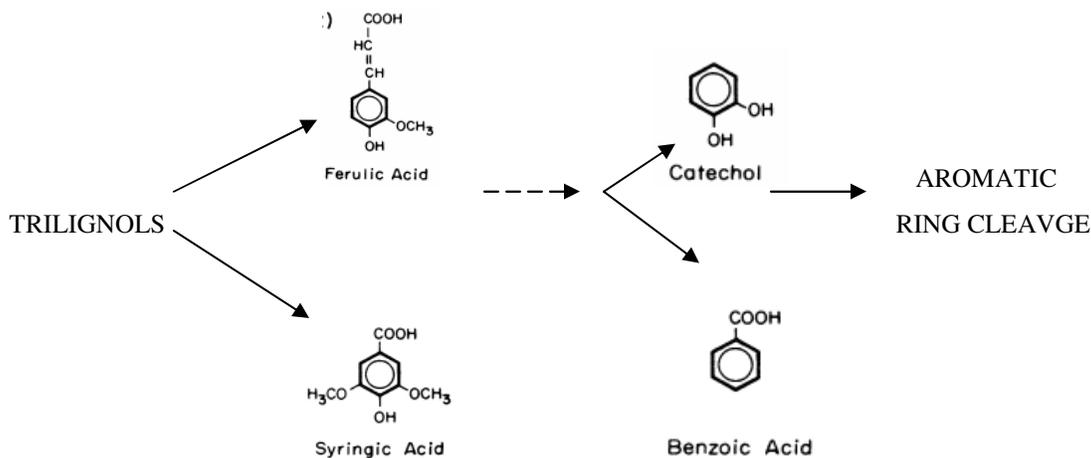


Figure 1.7: Schematic of lignin-derived substrate (trilignols) anaerobic biodegradation with the release of the lignin monomers proposed by Colberg and Young (*et. al.*, 1985).

Studies of anaerobic lignin biodegradation are generally conducted using anoxic sediment microflora, rumen or anaerobic sludge microorganisms. Lignin component of LCCs from grass, hard wood, and synthetic lignin were shown to be anaerobically mineralized by sediment microorganisms with the highest percentage of mineralization equal to 16.9 in the grass samples after 294 days of incubation (Benner *et. al.*, 1984). Lignin monomers were completely anaerobically mineralized by the marsh sediment inoculum (Phelps and Young *et. al.*, 1997). Another laboratory study investigated that acclimated enrichment cultures originated from anaerobic digester sludge showed up to 32 percent bioconversion of lignin oligomers of average MW 600 to methane and carbon dioxide (Colberg and Young *et. al.*, 1985). Moreover, the rate of anaerobic biodegradation of lignin fragments – oligomers is highly dependent on the molecular size and degree of polymerization of the substrate (Benner and Hodson *et. al.*, 1985).

Lignin hydrolysis is found to depend on temperature. Benner and Hodson (*et. al.*, 1985) showed that thermophilic (55°C) conditions enhance rates of lignin (pine lignin and synthetic lignin) and polysaccharides bioconversion to methane and carbon dioxide. Another study, of pulp liquor (12.9 percent of lignin and 30 percent of sugars)

degradation by anaerobic sludge bacteria at pH 7, showed an increase of water soluble lignin concentration as temperature increased from 35°C to 65°C. Elevated temperatures also affected hydrolyzed lignin bioavailability for further mineralization. Moreover, thermophilic conditions (65°C) provided the rate of lignin removal as high as 59% (Tartakovsky *et. al.*, 2003).

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## **CHAPTER 2: DEGRADATION OF LIGNOCELLULOSE AND SYNTHETIC LIGNIN AFFECTED BY THE ELEVATED TEMPERATURES**

### **ABSTRACT**

Lignin is known to be a recalcitrant material in conventional landfills. As bioreactor landfill technology becomes more popular, the effects of key elements of this technology such as elevated temperature, moisture and anaerobic digested sludge application on lignin degradation was investigated in this study. Lignin solubilization, production of lignin monomers, volatile fatty acids and biogas generation represent steps confirming lignin degradation in the anaerobic environment. These were analyzed for thermally pretreated reactors containing either synthetic lignin or newspaper (naturally occurring lignin) as a substrate. As a result of thermal pretreatment at 95°C, solubilized lignin containing the largest and smallest molecular sizes (more than 10 kDa and oligomers of less than 1 kDa relatively) were detected together with lignin monomers (syringic, ferulic, benzoic acids and catechol), suggesting that higher the temperature and moisture renders lignin more bioavailable for degradation. Lignin from newspaper was solubilized by 95°C exposure for 48 hours at an 8-fold higher concentration than newspaper lignin exposed to ambient temperature (23°C). However, there was no significant effect of thermal pretreatment for synthetic lignin. Both synthetic and newspaper lignin had similar patterns for soluble lignin during anaerobic incubation at 37°C using anaerobically digested sludge as an inocula. The soluble lignin of molecular size between 1 and 10 kDa was rapidly degraded into oligomers. The oligomers were then further degraded into lignin monomers. Another positive effect of elevated temperature was observed during the anaerobic incubation with release of 2 and 17 times more of total lignin monomers as organic carbon per gram of lignin substrate by preheated reactors compared to non preheated reactors filled with synthetic lignin and newspaper relatively. Moreover synthetic lignin reactors produced biogas which comprises approximately 6% percent of lignin carbon mineralized by the day 100 of incubation. An effect of temperature alone on the solubilization of lignin monomers was investigated. An

exponentially increasing trend for lignin monomers solubilization as a function of temperature was observed for three types of substrate, synthetic lignin, cardboard, and newspaper with the highest rate of solubilization for newspaper. Hence, these results support the hypothesis that high temperature and moisture, typical for bioreactor landfills, enhances lignin biodegradation under landfill conditions.

## **INTRODUCTION**

The growing mass of refuse produced by modern society is of an increasing concern to governments, communities and environmentalists. Since a major part of MSW consists of organic carbon, possible ways for mineralization of this carbon source have been extensively investigated in recent decades. Biodegradation of the organic part of refuse occurs at landfills, composting facilities and in anaerobic digesters.

Paper, wooden products, and other plant-originated materials are primarily composed of lignocellulose, lignin, cellulose and hemicellulose. Cellulose is the most abundant carbon source on earth and lignin is number two. Moreover lignin is the most abundant natural source of aromatic compounds. Completely hydrolyzed cellulose releases a simple sugar, glucose. Cellulose and hemicellulose are known to undergo nearly complete mineralization under anaerobic conditions. However, lignin is difficult to access for microorganisms and this restricts the biological availability not only of lignin, but also of hemicellulose and cellulose.

Lignin is considered to be recalcitrant in conventional landfills. It has a complex structure of randomly bonded highly branched polymers with abundant aromatic compounds which makes lignin degradation a rate-limiting step in the decomposition process. Additionally, lignin in paper products is an inhibiting agent for degradation in landfills because some of lignin monomers are toxic, eg.: phenols. However, in recent studies the lignin monomers have been shown to undergo complete methanogenic degradation using enriched cultures originated from anaerobic digester sludge (Young and Frazer *et. al.*,

1987). Another factor associated with lignin recalcitrance is its insolubility (Field *et. al.*, 2002). However, elevated temperatures (thermophilic temperatures of 55° to 65°C) have been shown to increase lignin solubilization which allows more rapid biodegradation (Benner and Hodson *et. al.*, 1985; Tartakovsky *et. al.*, 2003). Moreover, an anaerobic bacterial consortia showed an ability to hydrolyze lignin (Young and Fraser *et. al.*, 1987). After lignin is solubilized, it can undergo further microbial decomposition and partial mineralization.

Laboratory studies of lignin metabolism have revealed the pathways of its degradation. First, the heavy highly branched lignin polymeric molecule is decomposed into oligomers (dimers and trimers). The oligomers are further degraded into aromatic monomers. Young and Frazer (*et. al.*, 1987) researched the steps of oligomer degradation and showed that coniferil alcohol, vanillin, ferulic and syringic acids are typically released at the beginning of oligomer degradation. As the decomposition proceeds, benzoic acid, catechol, phenol, and resorcinol are formed. The next degradation step is aromatic ring fission and, as a result, carboxylic acids formation. Long chain carboxylic acids are further degraded to volatile fatty acids (VFAs) and hydrogen, which are mineralized to carbon dioxide and methane. In other studies it was found that the size of lignin fragments or molecular weight (MW) of hydrolyzed lignin affects the rate of anaerobic degradation. The highest rate of mineralization to CO<sub>2</sub> and CH<sub>4</sub> was observed for lignin of average MW of 200 or less. Less gas was produced from lignin fragments of average MW of 600-700, and the lowest rate was attributed to lignin of MW more than 1200 (Young and Frazer *et. al.*, 1987). Hence, the MW of partially hydrolyzed lignin plays a key role in the bacterial metabolism of lignocellulotic carbon.

One of the approaches to monitor lignin biodegradation intermediate byproducts under anaerobic conditions is to inhibit methanogens and allow intermediates to accumulate. The compound 2-bromo-ethane-sulfonic acid (BESA) suppresses methane formation. BESA is an analog of coenzyme M which controls methyl transfer for methanogens. BESA, when applied in sufficient concentration, was found to completely prevent methane formation (Colberg and Young *et. al.*, 1984). In the batch studies of Colberg and

Young (1984), a lignin-containing substrate of about MW 600 was inoculated with BESA amended cultures derived from anaerobically digested sludge. BESA at  $10^{-4}$  M was applied to provide complete methane inhibition for two years of incubation. As a result, solubilized lignin monomers (eg.: benzoic, ferulic acids) were present at about 10 times higher concentrations compared to BESA unamended samples. Moreover, a greater amount of a substrate was consumed and more VFAs were accumulated in BESA amended samples compared to reactors without BESA.

In this study, lignin solubilization, the presence of lignin monomers, VFAs and biogas formation were monitored to provide a clearer picture of the steps of lignin anaerobic biodegradation. Synthetic lignin and natural lignin from newspaper are believed to undergo anaerobic decomposition at mesophilic temperatures when seeded with mesophilic digested sludge. The application of sludge was chosen because of the positive effect of sludge seed shown in several lignocellulose degradation studies (Colberg and Young *et. al.*, 1985; Grbic-Galic *et. al.*, 1985; Kim *et. al.*, 1997; Clarkson and Xiao *et. al.*, 2000; Tartakovsky *et. al.*, 2003). Due to the limited time of the duration of experiment, it was decided to increase initial lignin bioavailability. Some of the reactors were exposed to a temperature of 95°C before inoculation as a pretreatment. Unheated reactors are used to compare the effect of lignin thermal solubilization on the biogas production rates and other indicator parameters of degradation. The significant accumulation of degradation byproducts in the presence of BESA was previously investigated by Colberg and Young (*et. al.*, 1984). Since little concentration of lignin monomers is expected in this study, some reactors are spiked with  $10^{-4}$  M of BESA to increase the intermediate concentrations.

An unexpected release of lignin monomers after thermal pretreatment prompted a study of the degree of aromatic compound release resulted from heating of the lignin-containing substrate without any microbiological activity present. Heating is a naturally occurring process affecting refuse biodegradation at landfills. For instance, anaerobic bioreactor landfills with enhanced biodegradation have temperatures up to 160°F (71°C)

(Pacey, 2001). Hence, the effect of temperatures from 23° to 65°C on lignin monomer release was analyzed.

## **MATERIALS AND METHODS**

### **PART A: Effect of temperature on the anaerobic biodegradation of lignin**

#### *Experimental design*

Two types of lignin-containing materials were chosen as a substrate for lignin anaerobic degradation studies. Synthetic lignin was used as a positive control. It contains 100 percent of hydrolytic lignin and does not contain other fiber constituents typical for naturally occurring lignin such as cellulose and hemicellulose which are located at the outer layer of the plant tissue and are partially degraded before lignin (Boda, 2002). Newspaper was chosen as the second material to study because it is abundant in landfills and contains natural lignin. Newsprint contains a large amount of lignin from wood pulp as opposed to office paper which contains a little lignin (Barlaz *et. al.*, 1997). Moreover it was shown in previous studies that newspaper reached 32-41 percent of potential chemical oxygen demand (COD) bioconversion in 300 days of anaerobic incubation, and office paper had a potential COD bioconversion of 71-85 percent in 165 days under the same conditions of incubation (Clarkson and Xiao *et. al.*, 2000). These results suggests that newsprint is more recalcitrant than office paper and results of lignin degradation in this study might indicate that other paper materials would be degraded to a greater extent than newspaper under the same conditions.

Reactors were filled with approximately 0.5 g of substrate in 30 mL of nanopure water according to the design matrix shown in Table 2.1, sealed, and exposed to the range of temperatures as a pretreatment stage. Then the reactors were inoculated with the reduced media solution containing seed, nutrients, and resazurin (an indicator of the reduced conditions). Some reactors are also spiked with BESA to inhibit methanogenic activity

and to accumulate more lignin degradation intermediates. After inoculation, the total volume of liquid in the reactors was 50 mL.

Operational conditions were set up to enhance degradation and provide results within 100 days of incubation. Lignocellulosic materials are degraded at landfills over a range of temperatures which are typically from 20°C to approximately 65°C (Gurijala *et. al.*, 1993; Pacey, 2001; Zhao, 2003). Ninety five °C was chosen for thermal pretreatment of reactors in order to provide an enhanced lignin solubilization at a shorter period of time, simulating the effect of elevated temperatures at landfills alone (without microbiological impact), and ambient temperature, 23°C, was chosen to simulate non-bioreactor landfill conditions. All reactors were thermally pretreated at 95°C or 23°C for 48 hours in a water bath before anaerobic inoculation.

Reactors were inoculated with the media solution and anaerobically incubated at 37±1°C until sampled. Mesophilic incubation temperature was chosen to reduce the temperature stress for the seed, which was obtained from a anaerobic mesophilic digester.

Five sampling periods were chosen: initial (day 1 of incubation), day 20, day 40, day 60 and day 100. Reactors were made in triplicates for each sampling period to provide representative statistical analysis. Reactors without substrate, but inoculated and exposed to all stages of the experiment were used as negative controls to eliminate the seed effect on the results.

### *Materials*

*Substrate.* Hydrolytic lignin with a carbon content of 50% - 67%, from Aldrich Chemical Co. (Figure 2.1) was used as a synthetic model compound containing 100 percent lignin. Newsprint of the Collegiate Times and Roanoke Times with 32.7 % lignin was shredded into strips by an office shredder, dried at 105°C over night to eliminate moisture, and ground to a powder using a Thomas Intermediate Wiley Mill with a 10-mesh screen.

*Inocula.* Anaerobically digested sludge was used as a seed and was obtained from a laboratory mesophilic anaerobic digester operated at  $37\pm 1^\circ\text{C}$ . Sludge was applied within 24 hours after it was obtained from the digester and kept at  $37^\circ\text{C}$  before usage. Prior to addition of the inocula, it was purged with nitrogen gas for about 1 hour before inoculation to reduce the amount of oxygen introduced into reactor.

*Nutrient solution.* The nutrient solution composition was adopted from Clarkson and Xiao (*et. al.*, 2000). All components were dissolved in distilled water (Table 2.2), mixed with a magnetic stirrer and purged with nitrogen gas for 2 hours to eliminate the oxygen in solution. Then solution was stored at  $4^\circ\text{C}$  for 12 to 48 hours before usage. Resazurin was added as an indicator of reduced conditions; it turns pink when the solution is reduced (Clarkson and Xiao *et. al.*, 2000), then it becomes colorless when anaerobic conditions are established. BESA at  $10^{-3}$  M was added to the solution for the BESA amended reactors.

### *Equipment*

Clear glass Wheaton 125 mL serum bottles were used as reactors. For thermal pretreatment they were filled with substrate and nanopure water, then sealed with rubber stoppers and aluminum crimps. For inoculation, a No.18 needle was used to inject the media solution into sealed bottles.

### *Sampling procedure*

Five sampling periods were chosen with an initial (day 1 of incubation), day 20, day 40, day 60 and day 100 sampling period. On the sampling day, a set of randomly chosen reactors (Table 2.1) were tested for biogas formation. Then sacrificial sampling was performed because the entire content of the reactors was required to run analysis for lignin degradation intermediates. Bottles were opened and the pH measured to check solution buffer capacity and the pH was adjusted to neutral if needed. Neutral pH is required for the experiment to provide proper conditions for methanogens (Grady, 1999). The actual pH ranged from 6.8 to 8.2 during the experiment and no pH adjustments were needed. The liquid part was further centrifuged at  $7,000 \times g$  (gravity acceleration,  $g = 9.81$

m/s<sup>2</sup>) for 30 minutes. Supernatant was filtered through 0.45 µm size filter paper. Part of the sample to be tested for particle size distribution (ultrafiltration) was stored at 4°C for a maximum of 24 hours. The rest of the sample was frozen for storage.

### *Analytical methods*

#### *Lignin hydrolysis*

The concentration of hydrolyzed lignin was measured as water soluble lignin (WSL) using the method developed by Orsa and Holmbom (*et. al.*, 1994). This procedure is chosen because of its relative ease and time efficiency. However, the weakness of this procedure is that up to 20 percent of the measurement is attributed to some dissolved and colloidal substances remaining in the water phase mainly represented by polysaccharides (Orsa and Holmbom *et. al.*, 1994). Lignin solubilization from reactors containing nanopure water was measured as mg of water soluble lignin per liter (WSL, mg/L). Precentrifuged frozen samples were used for the WSL determination after defrosting. Three milliliters of sample were transferred to 8 mL glass vials with a teflon liner in the cap, the pH was adjusted to approximately 3.5 pH with 30% H<sub>2</sub>SO<sub>4</sub>, then 1.5 mL of methyl tert.-butyl ether (MTBE) was added, vigorously shaken for 1 minute and centrifuged at 300g for 5 min. The MTBE layer on top of the sample was removed. Then MTBE extraction was repeated 2 more times. The water sample was diluted with deionized water at a 1:4 ratio and ultraviolet absorption was measured with a Beckman DU 640 spectrophotometer at wavelength 280 nm. Nanopure water was used as a reagent blank and to zero the instrument. Kraft lignin in methanol:nanopure water (80:20 v/v) was used for the quantitative calibration. The calibration curve had a goodness of fit with  $R^2 = 0.9928$ . Two replicates were used for blank, samples and calibration.

#### *Water soluble lignin molecular size distribution*

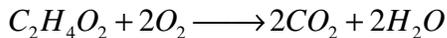
WSL concentrations when samples are separated into three molecular size fractions. Size fractions of less than 1 kDa (Kilodalton), between 1 and 10 kDa, and more than 10 kDa were obtained by ultrafiltration in stirred cells under 55 psi pressurized nitrogen flow through 1,000 and 10,000 nominal molecular weight limit (NMWL) membranes (Ultracel Amicon YM-1, Millipore Inc.). The filtrate was analyzed for each size fractions.

### *Analysis of lignin monomers with capillary gas chromatography*

Capillary gas chromatography (GC) was used for aromatic lignin monomers analysis. Ferulic, syringic, benzoic acids and catechol are lignin bidegradation intermediates and typical model compounds for lignin monomers degradation studies (Grbic-Galic *et.al.*, 1985; Phelps and Young *et. al.*, 1996). The extraction - derivatization procedure was adopted from Colberg and Young (*et. al.*, 1985). Two milliliters of sample, transferred into 8 mL glass vials, were acidified with two drops of 50% (v/v) sulfuric acid. They were then extracted with 1 mL of methylene chloride (anhydrous, 99.9%, Fisher Scientific, Inc.). Vials were vigorously shaken and the layers allowed to separate. Two hundred  $\mu\text{L}$  of methylene chloride were added to 2 mL GC autosampler vials and dried under nitrogen. Two hundred  $\mu\text{L}$  of Regisil RC-2 (Bis(trimethylsilyl)trifluoroacetamide +1% Trimethylchlorosilane, Regis Technologies Inc.), were added to vials with a teflon-coated septum. The samples were mixed using a vortex mixer for 20-30 seconds, then 200  $\mu\text{L}$  of acetonitrile (HPLC grade, Fisher Scientific, Inc.) was added and mixed using a vortex mixer. Then samples were heated to 60°C for 10 minutes. When the samples cooled down, they were analyzed using a capillary GC. Nanopure water was used as a reagent blank. All samples, standards, and blanks were run in duplicates. Control lignin values were subtracted to eliminate seed interference.

### *Volatile fatty acids analysis*

Samples were thawed before analysis. Four standards were prepared using a Supelco Standard Fatty Acid Mix for acetic, propionic, butyric, iso-butyric, n-valeric, iso-valeric, and caproic acids. Nanopure water was used for the blank and treated it the same way as the samples. A volume of 0.990 mL of precentrifuged filtered samples were transferred to a in 2 mL GC autosampler vial and acidified with 0.010 mL of 85% o-phosphoric acid. Samples were analyzed using a Shimadzu GC 14A with a flame ionization detector (FID) column. Samples were analyzed in triplicates with nanopure water used as reagent blank, and results for the controls were subtracted to eliminate seed interference. Concentrations of VFAs are reported as total VFAs, which is the sum of equivalent acid concentrations. Acids in mg/L are converted into mg COD/L using the following approach:



Hence,

$$\frac{2 \text{ mole } O_2}{1 \text{ mole acetat}} \times \frac{32 \text{ g } O_2 / \text{ mole } O_2}{60.053 \text{ g acetat} / \text{ mole acetat}} = 1.07 \text{ gCOD} / \text{ g acetate}$$

$$\text{Conc. acetat} (\text{mg} / \text{L}) \times 1.07 \text{ mgCOD} / \text{mg acetat} = \text{conc mgCOD} / \text{L}$$

### *Biogas production*

Biogas production was monitored for each sampling period to determine the progress of anaerobic degradation. In this study methane was only partially inhibited in the BESA amended reactors. Hence, increasing trends of CH<sub>4</sub> generation for reactors of all types were measured as well as CO<sub>2</sub>. The accumulated pressurized biogas was released from reactors into 1 L gas-tight Tedlar bags. The volume of gas was measured from the Tedlar bag with the 20 mL syringe. The gas composition was tested by the immediate injection of 0.5 mL of biogas sample from the headspace of the reactor into sampling port of the Shimadzu GC 14A with thermal conductivity detector (TCD). High purity gas standards were run for calibration.

## PART B: Temperature effect on the lignin monomers solubilization

### *Experimental design*

Synthetic lignin and newspaper substrates showed a release of lignin degradation intermediates after thermal pretreatment (no microbial exposure) in Part A of the study. Hence, the amount of monomers solubilized with an increase of the exposure temperature was studied. It was previously documented that high temperatures affect the amount of lignin solubilized, with a distinctive linear trends for seeded reactors (Tartakovsky *et. al.*, 2003), and exponential trends for physical exposure (Irani, 2006). Thus, synthetic lignin representing 100 percent of purified lignin substrate and newspaper, the highest lignin containing paper, was chosen for this study. It was decided to include cardboard because it is also high in lignin content (20.3 % was reported by Barlaz *et. al.*, (2006)), and it is a

typical component of municipal solid waste found at landfills (Micales and Skog *et. al.*, 1997).

Reactors were filled with approximately 2g of substrate and 100 mL of nanopure water, and exposed to the following range of elevated temperatures: 23, 37, 45, 55, and 65°C for 48 hours. The samples were cooled and the supernatant filtered through a 0.45 µm pore-size filter. The filtrate was analyzed for the presence of lignin monomers. To ensure no microbiological activity, all reactors are spiked with 1% of Clorox® Bleach solution (1 mL of 1:100 of Clorox® Bleach in distilled water per reactor). All samples are prepared in triplicate to provide a reliable statistical analysis.

### *Materials*

Hydrolytic lignin from Aldrich Chemical Co. was used as a synthetic model compound. Newsprint and cardboard were shredded by an office shredder, dried in 105°C oven over night to get rid of moisture, and ground to a powder using a Thomas Intermediate Wiley Mill with a 10-mesh screen.

### *Equipment*

The same equipment as for Part A is used: Wheaton 125mL serum bottles were used as reactors, and sealed with rubber stoppers and aluminum crimps.

### *Analytical methods*

#### *Analysis of lignin monomers with capillary GC*

The same procedure as for Part A of the experiment was followed.

## RESULTS AND DISCUSSION

### PART A: EFFECT OF TEMPERATURE ON THE ANAEROBIC BIODEGRADATION OF LIGNIN

#### *2.1 Water solubility of lignin enhanced by elevated temperatures under anaerobic conditions*

The first step of lignin decomposition is when the solid lignin (fiber material) is hydrolyzed. In this study the effect of two major factors inducing hydrolysis were studied, solubilization due to thermal exposure (Tartakovsky *et. al.*, 2003, Irani, 2005) and microbiological solubilization (Young and Frazer *et. al.*, 1987). Moreover, the hydrolysis of polymeric lignin molecules results in the release of lignin fragments of different size. The profile of the size distribution of lignin fragments was investigated for reactors with different lignin sources and pretreatment over 100 days of 37°C incubation. WSL with molecular weights less than 1,200 are known to undergo complete mineralization with acclimated digested sludge cultures as inocula (Young and Frazer *et. al.*, 1987).

#### *Synthetic lignin solubilization*

In this part of the study the effect of temperature on the pattern of synthetic lignin solubilization was investigated. The highest percentage of lignin solubilization by thermal pretreatment is observed in the 95°C pretreated reactors and yielded 0.44 percent of the initial lignin substrate. However, a relatively similar percentage of lignin, 0.38%, was solubilized in the 23°C pretreated reactors. This may be explained by the properties of the substrates. Synthetic lignin does not contain other fibrous constituents, such as cellulose and hemicellulose fibers, which may affect lignin solubilization. Hence, the effect of elevated temperature on the release of solubilized lignin polymers is relatively insignificant for the synthetic model compound.

After inoculation, all reactors were incubated at 37°C until sampled. The double effect of thermal and microbiological lignin hydrolysis was expected. As incubation proceeded the WSL concentrations were measured (Figure 2.2). The WSL for all of the conditions and substrates (95°C pretreated with and without BESA amended inocula, 23°C pretreated with BESA amended inocula) remained at a relatively constant level. The slightly higher level of WSL in the 23°C pretreated reactors after day 40 may be due to variability in the sample analysis.

A more detailed insight to the lignin hydrolysis is presented in the following section by analysis of the molecular size distribution of WSL.

► ***Molecular size distribution of WSL***

As lignin hydrolyses it releases lignin polymeric molecules of different size, which have differing degrees of bioavailability for microorganisms (Young and Frazer *et. al.*, 1987). Solubilization of three fractions of WSL molecular size was studied; polymers more than 10 kDa, medium size (between 1 and 10 kDa), and oligomers (less than 1 kDa).

***23°C pretreated reactors amended with BESA.*** In reactors with synthetic lignin as a carbon source, on the first day of incubation a higher concentration of WSL was measured for lignin of more than 10 kDa molecular size, which comprised 48 percent of total WSL. A smaller concentration of lignin was released in the size between 1 and 10 kDa (about 38 percent), and the least amount present (about 18 percent) was of less than 1 kDa lignin (Figure 2.3). As incubation proceeded and the inoculated reactors were exposed to 37°C, the overall pattern of WSL molecular size distribution (Figure 2.3) showed that medium size lignin molecules were primarily degraded. As a result, the biggest and smallest fractions (more than 10 kDa and less than 1 kDa relatively) increased while the degradation of the medium size fraction occurred. After day 40, when medium size WSL was depleted, the decomposition of the larger polymeric fraction occurred, reducing its concentration in the solution and producing medium sized lignin by day 60. The above observations suggest that depolymerization of solubilized lignin occurred at a faster rate than lignin monomer generation, since no significant decrease in

the smallest monitored WSL fraction was observed. This observation is similar to results by Colberg and Young (*et. al.*, 1985).

**95°C pretreated reactors.** There was no significant difference observed in total WSL released after thermal pretreatment for the 23°C and 95°C pretreated reactors. However, the molecular size distribution profile (Figure 2.4) at day 1 does show a difference in the effect of the temperature of pretreatment. A higher concentration, 15.11 mgWSL/L, of the small oligomeric fraction was observed for the 95°C pretreated reactors compared to 6.99 mgWSL/L for the same fraction present in the unheated reactors (Figure 2.3). This indicates a release of greater quantities of more bioavailable lignin under high temperature. Unfortunately, only separation into less than 1 kDa and more than 1 kDa fractions was feasible for day 1 for preheated reactors so the size ranges were determined only for more and less than 1 kDa (Figure 2.4).

As the incubation proceeded, a pattern similar to 23°C pretreated reactors for WSL distribution was observed. The big polymeric fraction is present at higher concentrations than other fractions (Figure 2.4). The medium molecular size (between 1 and 10 kDa) lignin is converted into the oligomeric fraction and not observed at later dates of incubation. The less than 1 kDa WSL is present at relatively constant concentration of approximately 10 mgWSL/L for both BESA amended and unamended reactors even though the medium size lignin fraction decreases with time. This indicates the further degradation of lignin into its intermediates and finally into gas.

### *Solubilization of lignin from newspaper*

In this part of the study the effect of temperature on the pattern of newspaper lignin solubilization was investigated. Reactors, pretreated at 95°C for 48 hours in a hot water shaker bath before the seed was added yield 0.32% solubilization of initial solid lignin originating from newspaper. Reactors not exposed to elevated temperature and pretreated at 23°C, showed that only 0.04% of lignin is present in the aqueous phase before anaerobic incubation. This is approximately 8 times greater WSL concentration for preheated reactors compared to the unheated. Hence, thermal pretreatment provides a

greater amount of a hydrolyzed lignin in a form which is potentially more bioavailable for microorganisms to utilize as a carbon source when the substrate contains lignocellulotic and other materials.

The highest concentrations of WSL during incubation were for the 95°C preheated reactors with BESA amended cultures, and the lowest concentrations were observed for the 23°C pretreated reactors (Figure 2.5). Lignin solubilization proceeded with similar trends for all three types of reactors. It levels off by the end of the incubation. The greater amount of WSL in reactors with BESA amended cultures indicates the effect of BESA was partial inhibition of methanogenesis which influences degradation byproduct accumulation.

► ***Molecular size distribution of WSL***

Reactors with newspaper as a carbon source demonstrated similar molecular size distribution patterns of lignin hydrolysis to reactors with a synthetic lignin as a substrate. The initial concentration of lignin in newspaper is less than in synthetic lignin (newspaper consists of approximately 32.7% of lignin fibers). This results in generally lower concentrations of all fractions of WSL measured for newspaper (Figure 2.4, 2.7).

***23°C pretreated reactors amended with BESA.*** At day one, only the polymeric fraction of WSL of more than 10 kDa molecules was present (Figure 2.6). As the degradation continued, this fraction decreased slightly and the less than 1 kDa fraction was formed. This is an indicator of further solubilization of solid lignin and of the conversion of bigger lignin molecules into smaller ones. At day 40, all the fractions of WSL were present, with more WSL of bigger size, less of medium, and the least of less than 1 kDa. Moreover, the fraction of less than 1 kDa decreased which indicates that it has been degraded. By day 60 of incubation the small fraction is completely degraded and only the 1 to 10 kDa lignin fragments are present. By the day 100, some of the solubilized lignin of 1 to 10 kDa size is decomposed and the less than 1 kDa fraction shows up. No WSL larger than 10 kDa fraction was measured, suggesting that no significant solubilization of

newspaper occurred and that the middle fraction is further depolymerized into less than 1 kDa.

**95°C pretreated reactors.** Patterns for the molecular size distribution of fractions of WSL for preheated reactors (Figure 2.7) have similar trends as other types of reactors (Figures 2.3, 2.4, 2.6). The big polymeric fraction is slowly converted into the medium molecular size (between 1 and 10 kDa) lignin fraction, which is readily converted into oligomers (WSL of less than 1 kDa). The rate of medium fraction depolymerization is faster than degradation of the oligomeric fraction, which is observed through all the experiment for both reactors with BESA amended and unamended cultures (Figure 2.7). Only separation into less than 1 kDa and more than 1 kDa fractions was feasible for day 1 of preheated reactors so the size ranges were determined only for less and more than 1 kDa (Figure 2.7).

## 2.2 Lignin monomers anaerobic decomposition and fate

Lignin anaerobic degradation pathways have been studied by many researchers (Colberg and Young *et. al.*, 1985; Garbic-Galic *et. al.*, 1985; Young and Frazer *et. al.*, 1987; Colberg *et. al.*, 1988). As solid lignin fibers are solubilized, lignin polymers are further decomposed into lignin trimers and dimers, which are composed of three and two aromatic compounds (lignin monomers). Then monomers are formed. Within lignin monomers it has been found that coniferyl alcohol, vanillin, ferulic and syringic acids are primary intermediates that are formed, and then benzoic acid, catechol, resorcinol and phenol were detected before aromatic ring cleavage (Young and Frazer *et. al.*, 1987). Hence, measured concentrations of ferulic and syringic acid indicate lignin depolymerisation in this study, and benzoic acid and catechol concentrations denote that the carboxylic acids will be formed next.

### *Synthetic lignin monomers degradation*

In this part of the study the fate of synthetic lignin monomers was investigated. At first, lignin monomers concentrations were measured after thermal pretreatment and right after

inoculation (to provide consistent conditions for further data comparison), then measurements continued at each sampling period.

**23°C pretreated reactors amended with BESA.** Concentrations of syringic and ferulic acids as high as 1.9 and 4.0 mg of monomer per liter were measured at day 1 (Figure 2.8). This suggests that some aromatic compounds were released as a result of exposure of lignin to water at 23°C.

As anaerobic incubation proceeded, catechol and benzoic acid were formed and no increase in syringic and ferulic acid was observed (Figure 2.8). By day 40, all syringic and ferulic acids were metabolized. The catechol concentration increases by 4.86 mg/L (with  $\pm 4.69$  standard deviation) at day 40 and stayed approximately constant until the last day of incubation (day 100). However, the benzoic acid that accumulated by day 40 was all degraded by day 60 and not detected afterwards.

**95°C pretreated reactors.** Similar to the 23°C pretreated reactors, the pattern for lignin monomer degradation was observed for the 95°C pretreated reactors both amended with BESA (Figure 2.9,a) and unamended (Figure 2.9,b). Syringic and ferulic acids were present at the first day of incubation with concentrations as high as 10.8 and 4.7 mg of monomer per liter, respectively. This is approximately twice as much as it was measured for the 23°C pretreated reactors, confirming the effect of temperature on monomer release. Thus, 95°C temperature exposure affected the amount of primary intermediates released.

BESA was added to reactors at  $10^{-3}$  M concentration, but this was insufficient to completely inhibit methanogens. Methane was observed by day 20 (Figure 2.16 (a)) of incubation. Reactors with BESA amended seed and without BESA showed similar results for accumulation of lignin monomers (Figure 2.9). As incubation proceeded benzoic acid was formed, indicating lignin degradation. The highest concentration was produced by day 40 of incubation and reached 54.36 mg/L and 72.84 mg/L for BESA amended reactors and reactors without BESA, respectively.

Synthetic lignin used in this experiment typically contains 50-67% of organic carbon (Aldrich). If one assumes an average organic carbon portion of synthetic lignin is 58.5%, one can compare total lignin monomers generated during incubation (ferulic, syringic, benzoic acids and catechol) expressed as equivalent mg of organic carbon per g of lignin substrate (Figure 2.10). Reactors, which have been preheated, released much higher levels of total monomers over the incubation period, with the greatest difference at day 40. The preheated reactors without BESA released 2 times more lignin monomer (5.05 mgC/g lignin) than maximum amount of monomers released from non preheated reactors (total concentration 2.98 mgC/g lignin). However, after day 60, the total lignin monomer concentration decreased and continued to be relatively low (not more than 0.76 mgC/g lignin for 95°C pretreated reactors without BESA addition).

Total lignin monomers expressed as equivalent of organic carbon yields a maximum of 1.73 percent (for preheated reactors without BESA at day 40) of carbon degraded from synthetic lignin (Figure 2.11).

### *Newspaper lignin monomers degradation*

In this part of the study the fate of newspaper lignin monomers was investigated. Newspaper contains less lignin (about 32.7% of lignin) than a synthetic model compound (100% lignin), which may result in either release of lignin monomers at undetectable concentrations (below detection limit of the instrument) or their absence due to the immediate utilization of monomers by microorganisms.

**23°C pretreated reactors amended with BESA.** There was no syringic or ferulic acids measured during the experiment. Moreover, catechol and benzoic acid were first observed at day 20 of incubation (Figure 2.12). This correlates to the WSL size distribution profile for this type of reactors where only big polymeric (more than 10 kDa) lignin fraction was present in solution (Figure 2.6). As incubation proceeded oligomeric WSL fractions appear by day 20 as well as lignin monomers. Benzoic acid was degraded after day 60 and catechol was observed at higher concentration until the end of incubation

(Figure 2.12). This may indicate that benzoic acid is more readily biodegradable under methanogenic conditions, which was also observed by Garbic-Galic (*et. al.*, 1985).

**95°C pretreated reactors.** As opposed to the non preheated reactors, ferulic, benzoic acids and catechol were all detected at the first day of incubation of both types of 95°C pretreated reactors (Figure 2.13), indicating the effect of elevated temperature on the lignin monomers solubilization. As incubation proceeded, ferulic and syringic acids were depleted by day 60, and the benzoic acids and catechol concentrations increased (Figure 2.13). This can be due to the degradation of other lignin monomers. The highest concentration was observed for benzoate, 8.46 mg/L at day 100 for reactors without BESA. However, lignin monomers were present at lower concentrations compared to reactors with synthetic lignin as a substrate (Figure 2.9).

Newspaper used in this experiment is made of paper pulp which contains kraft lignin (Benner and Hodson *et. al.*, 1985). Kraft lignin typically contains 45-65% of organic carbon (Aldrich Inc.). If one assumes average organic carbon portion of newspaper lignin is 55%, one can compare total lignin monomers generated during incubation (ferulic, syringic, benzoic acids and catechol) expressed as equivalent mg of organic carbon per g of lignin substrate (Figure 2.14, a). Reactors, which have been preheated, released much higher levels of total monomers through the incubation with the greatest difference at day 20. The preheated reactors without BESA released 17 times more of lignin monomers (1.81 mgC/g lignin) than maximum amount of monomers released from non preheated reactors (total concentration 0.11 mgC/g lignin).

Total lignin monomers expressed as equivalent of organic carbon yield a maximum of 0.66 percent (for preheated reactors with BESA at day 40) of carbon degraded from newspaper lignin (Figure 2.14, b). Unheated reactors had much lower yield of lignin monomers release during the incubation with the highest value of 0.08% expressed as organic carbon (Figure 2.14, b) which is about 8 times less than from preheated reactors.

Lignin degradation for both synthetic and newspaper lignin was shown by the presence of lignin monomers during the anaerobic incubation. The positive effect of pretreatment at elevated temperature (95°C) expressed as an increase of accumulated monomers for 95°C pretreated reactors by day 40 compared to relatively constant low concentrations of monomers in 23°C pretreated reactors (Figure 2.10, 2.14).

### 2.3 Volatile fatty acid formation

Acetic, propionic, butyric, iso-butyric, iso-valeric, n-valeric, and capronic acids were monitored during the anaerobic incubation. These VFAs represent short-chain C<sub>2</sub> to C<sub>6</sub> carboxylic acids. Total VFAs were measured for all the reactors during the anaerobic incubation.

**23°C pretreated reactors amended with BESA.** There was some VFAs accumulation observed, with similar trends to the preheated reactors with BESA (Figure 2.15). VFA production from the synthetic lignin reactors (Figure 2.15) was monitored at low but significant rate through the incubation and was attributed to the lignin degradation alone.

**95°C pretreated reactors.** BESA amended reactors seem to be able to accumulate VFAs at a higher concentrations than reactors without BESA (Figure 2.15). Non BESA inhibited reactors show the fast depletion of total VFAs; no VFAs were measured after day 40. BESA amended reactors appear to reach this stabilization point only by day 100 of incubation. The overall profile of VFAs generation by the BESA amended reactors is similar to the VFAs generated by the refuse anaerobic degradation during 110 days in the Barlaz, *et.al.*, (1989) study.

Reactors with a newspaper as a substrate produced more total VFAs with a maximum of 4.46 mg COD/L for BESA amended cultures. Higher VFAs in newspaper reactors are due to the presence of the more biodegradable cellulose and hemicellulose. However, the VFAs production by reactors with synthetic lignin as a substrate have similar patterns to newspaper, which is attributed to the lignin degradation alone (Figure 2.15).

## *2.4 Biogas production during anaerobic incubation*

Biogas production from reactors was monitored during the anaerobic incubation. The amount of BESA was insufficient for complete inhibition of methanogens. Methane generation was observed in reactors of all types (Figures 2.16 (a), 2.17 (a)). However, partial inhibition of methanogens by BESA occurs and the non BESA amended reactors with both synthetic lignin and newspaper substrates produce more methane than reactors with BESA. Moreover, preheated reactors with BESA generated more methane over time compared to non-preheated (23C pretreatment) reactors with BESA (Figures 2.16 (a), 2.17 (a)).

Methane and carbon dioxide production observed from the reactors with synthetic lignin as a carbon source is due to the lignin mineralization by the digested sludge cultures. Unfortunately, it was impossible in this study to isolate the contribution of newspaper lignin into methane production and compare this rate to the rate of synthetic lignin mineralization. However, as high as 22.6 mL of methane (which comprises 44.6% of total biogas) was generated by the synthetic lignin alone by the preheated reactors without BESA as a result of 100 day of anaerobic incubation. Total biogas (methane and carbon dioxide) generated by preheated synthetic lignin reactors at day 100 of incubation comprise possibly 6% of carbon mineralized per g of lignin.

Carbon dioxide was monitored at approximately same level for reactors of all types. It levels off after day 20 (Figures 2.16 (b), 2.17 (b)).

### **PART B: TEMPERATURE EFFECT ON THE LIGNIN MONOMERS SOLUBILIZATION**

The unexpected release of lignin monomers in the Part A of this study led to the investigation of the temperature effect on the lignin monomer solubilization. It was previously observed that lignin is solubilized with an exponential dependence on

temperature (Irani, 2005). Lignin monomers are very numerous, but ferulic, syringic, benzoic acids and catechol are typical representatives for lignin degradation studies (Colberg and Young *et. al.*, 1985; Garbic-Galic *et. al.*, 1985; Young and Frazer *et. al.*, 1987; Colberg *et. al.*, 1988). Thus, the concentrations of these four compounds were studied for possible trends.

Synthetic lignin reactors released syringic and benzoic acids, and catechol. However, only benzoic acid and catechol were detected in newspaper and cardboard reactors. Lignin monomers were converted into equivalent units of mg organic carbon per gram of substrate using same assumptions made in Part A. Concentrations of total lignin monomers in the solution for reactors of all types increase exponentially (Table 2.3). Synthetic lignin has a rate 1.3 to 3.9 - fold higher concentration of total monomers solubilization (0.81 mgC/g substrate at 65°C). Newspaper (0.64 mgC/g substrate at 65°C) released higher levels of monomers compared to cardboard (0.21mgC/g substrate at 65°C) (Figure 2.18). This can be explained by the percentage of lignin in these materials; synthetic lignin is 100% lignin, newspaper is approximately 32.5%, and cardboard is only about 16.5% lignin. However, interestingly, when converted into percent of organic carbon in lignin, newspaper has the highest percentage of solubilization (0.35% at 65°C), followed by cardboard (0.23% at 65°C), and then synthetic lignin (0.14% at 65°C) (Figure 2.19).

Temperature solubilization of lignin monomers from a solid lignin source indicates that the high temperature – high moisture systems such as bioreactor landfills can provide some lignin degradation. This differs from conventional landfills where lignin is considered to be relatively inert. Hence, the approach of using the cellulose to lignin ratio (C/L) of refuse to estimate sanitary landfill stabilization may need revisions since C/L may not be accurate for bioreactors.

## SUMMARY AND CONCLUSIONS

In this study step by step lignin anaerobic degradation and mineralization were analyzed. Moreover, the release of lignin monomers as a result of exposure of elevated temperature on lignin containing substrate (assuming no microbiological activity) is investigated for possible trends. It was hypothesized that preheated lignin containing substrate would enhance the lignin anaerobic degradation by the digested sludge inocula. The anaerobic biodegradation of lignin monitored in this study may suggest revaluing of C/L, landfill stabilization parameter, as lignin may be not an inert material for new landfill technology such as bioreactors. As a result of this study the following conclusions are made:

- Temperature was found to highly affect lignin solubilization in water and enhance biological lignin solubilization by the anaerobic digested sludge inocula.
  - Newspaper released approximately 8 times more of water soluble lignin from preheated reactors compared to not preheated reactors before microbiological solubilization-degradation of lignin.
  - Elevated temperature alone affected the molecular size distribution of solubilized lignin polymers. When substrate was preheated, it released more WSL of the smallest molecular size tested. This lignin fraction is attributed to the lignin oligomers, which are known to undergo complete mineralization under anaerobic conditions in laboratory studies (Young and Frazer *et. al.*, 1987).
  - Hence, bioreactor landfills can provide more of a biodegradable lignin as a result of exposure of elevated temperatures and moisture to paper and other lignin containing refuse.
  
- Lignin biodegradation was observed to occur with the release and subsequent decomposition of lignin degradation by-products such as lignin monomers.
  - Reactors, which had been preheated released much higher levels of total monomers throughout the incubation. About 2 times more of total lignin monomers were released in preheated reactors containing synthetic lignin than

in non preheated reactors. In reactors with newspaper as a substrate, 17 times more of total lignin monomers were observed compared to non preheated reactors. Hence, elevated temperatures and moisture can enhance lignin monomers anaerobic degradation when the digested sludge present.

- Anaerobic sludge used as inocula was capable of lignin monomers degradation, including aromatic ring fusion. Hence, digested sludge disposal at landfills can provide not only another source to increase moisture content, but also an additional and effective source of microorganisms capable of recalcitrant lignin degradation.
- 
- VFAs and biogas produced from synthetic lignin prove lignin anaerobic degradation and mineralization is possible under laboratory conditions with the rate of mineralization of approximately 6% of carbon per g of lignin released as biogas by day 100 of anaerobic incubation from the initial synthetic lignin. This value is very approximate due to indirect estimate of the total organic carbon of lignin, but close to values of 2 to 4% percent of synthetic and natural pine lignin mineralization reported by Benner and Hodson (*et. al.*, 1985).
  - An enhancement effect of the elevated temperature on biogas production during the anaerobic incubation was observed.
  - Exponential dependence of lignin monomers solubilization upon the temperature increase was observed for three types of lignin source (newspaper, cardboard, and synthetic lignin):
    - Newspaper revealed the highest percentage of organic carbon solubilization in a form of lignin monomers among the tested materials.
    - Hence, temperatures typically occurring in bioreactor landfills can alone affect release of lignin monomers, which are even more prone to be biodegraded than solubilized lignin.

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## TABLES & FIGURES

Table 2.1: Design matrix: the set up of the study of the temperature effect on the anaerobic bioconversion of lignin (each type includes triplicates and represents amount per one sampling period).

Pretreatment temperature, °C	BESA amended inocula			inocula without BESA		
	Control (0% lignin)	Synthetic lignin (100% initially)	Newspaper (32.7% initially)	Control (0% lignin)	Synthetic lignin (100% initially)	Newspaper (32.7% initially)
95	3	3	3	3	3	3
23	3	3	3	--	--	--

Table 2.2: Macro and micronutrients for serum bottles reactors, adopted from Clarkson and Xiao (*et. al.*, 2000).

Constituent	Concentration, mg/L	Constituent	Concentration, mg/L
NaHCO <sub>3</sub>	6000	CoCl <sub>2</sub> *6H <sub>2</sub> O	0.5
NH <sub>4</sub> Cl	1200	CuCl <sub>2</sub> *2H <sub>2</sub> O	0.5
K <sub>2</sub> HPO <sub>4</sub>	500	ZnSO <sub>4</sub> *7H <sub>2</sub> O	0.5
MgSO <sub>4</sub> *7H <sub>2</sub> O	300	Na <sub>2</sub> SeO <sub>3</sub>	0.5
CaCl <sub>2</sub> *2H <sub>2</sub> O	100	MnCl <sub>2</sub> *4H <sub>2</sub> O	0.5
FeCl <sub>2</sub> *4H <sub>2</sub> O	40	H <sub>3</sub> BO <sub>3</sub>	0.5
KI	2	NiCl <sub>2</sub>	0.5
Yeast Extract	1000	NH <sub>4</sub> VO <sub>3</sub>	0.5

Table 2.3: Trends equations of lignin monomers solubilization with temperature exposure for 48 hours  
(includes goodness of fit coefficients).

Lignin source	Exponential trend equation	R <sup>2</sup>
Synthetic lignin	$TLM^* = 0.093e^{0.0364t^{**}}$	0.8267
Newspaper	$TLM^* = 0.0243e^{0.0498t^{**}}$	0.9942
Cardboard	$TLM^* = 0.0197e^{0.0335t^{**}}$	0.8643

\*TLM is total lignin monomers concentration, mgC/g substrate

\*\*  $t$  – temperature, °C

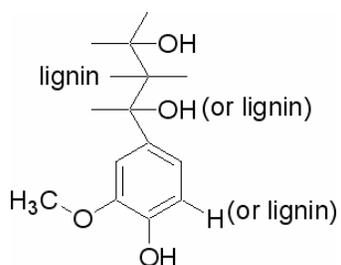


Figure 2.1: Schematic of hydrolytic lignin (by Sigma-Aldrich Inc.).

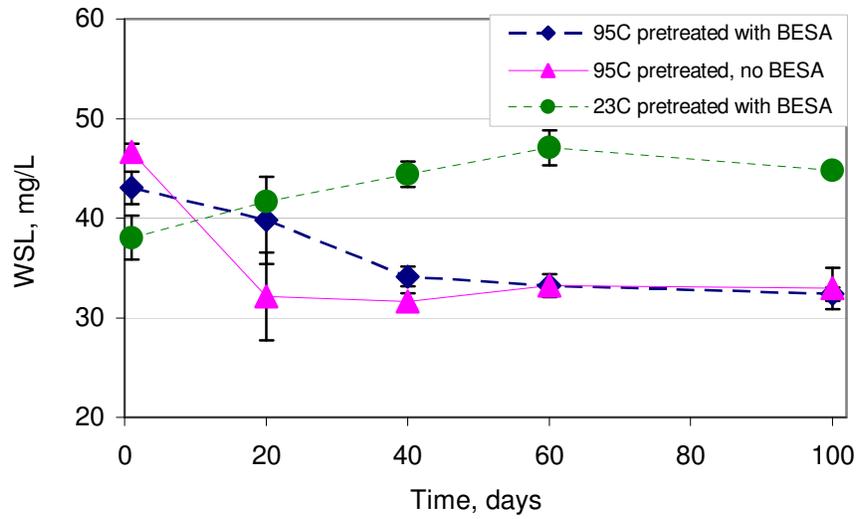


Figure 2.2: Synthetic lignin solubilization during the incubation of thermally pretreated reactors and inoculated reactors with and without BESA (with error bars indicating 95% confidence interval of mean).

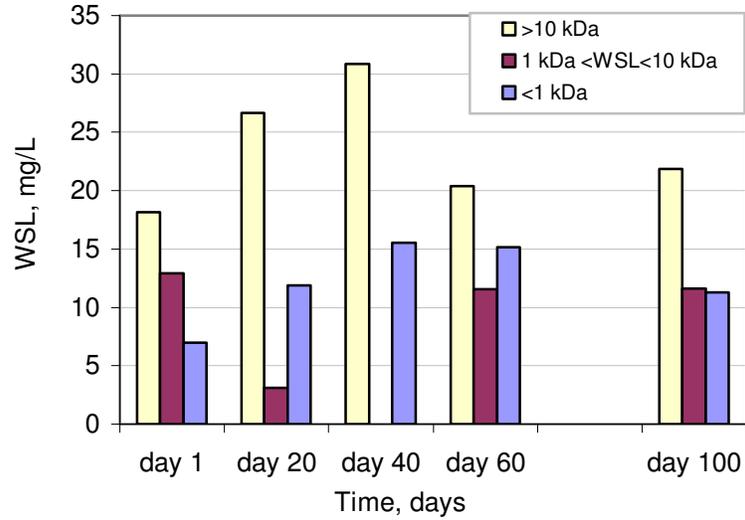


Figure 2.3: Water soluble lignin fragments size distribution for synthetic lignin reactors pretreated at 23C with partial BESA inhibition.

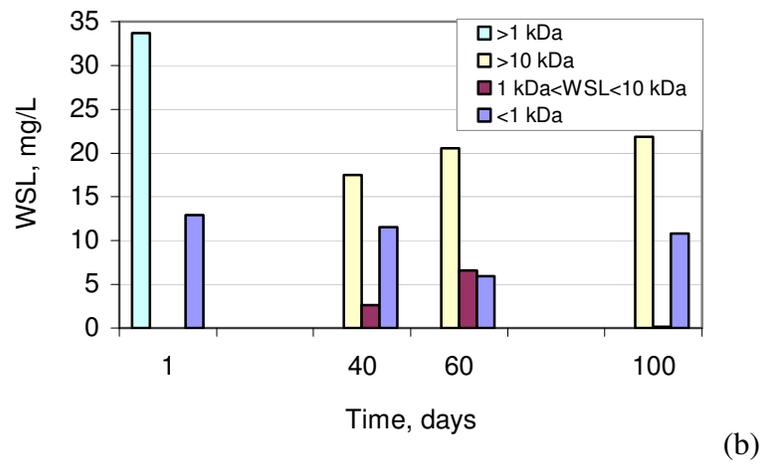
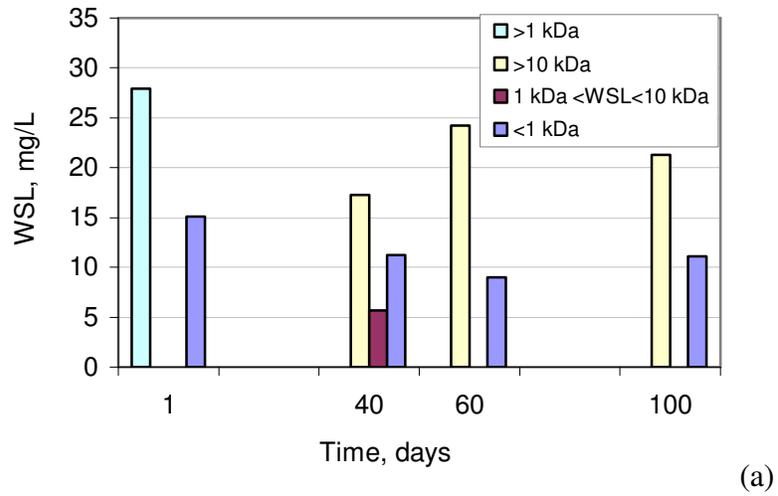


Figure 2.4: Water soluble lignin fragments size distribution for synthetic lignin reactors pretreated at 95°C with (a) and without (b) BESA amended inocula.

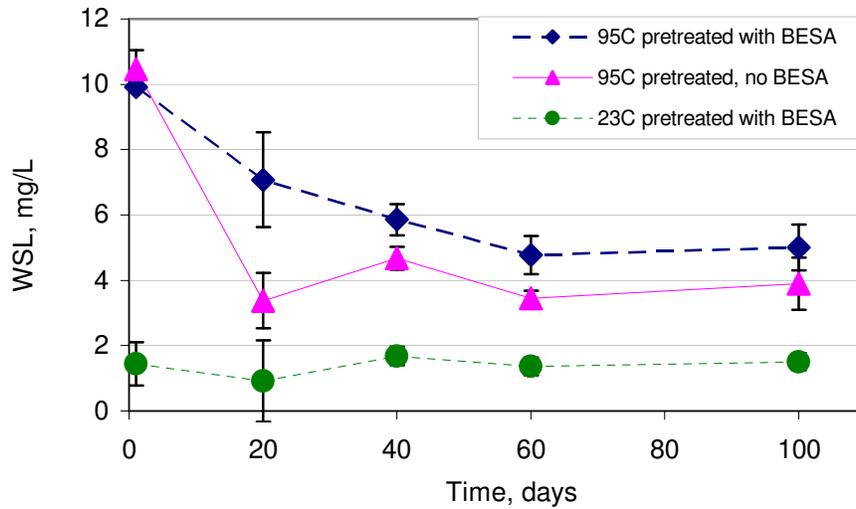


Figure 2.5: Newspaper originated lignin solubilization during the incubation of thermally pretreated reactors and inoculated afterwards with and without BESA amended cultures (with error bars indicating 95% confidence interval of mean).

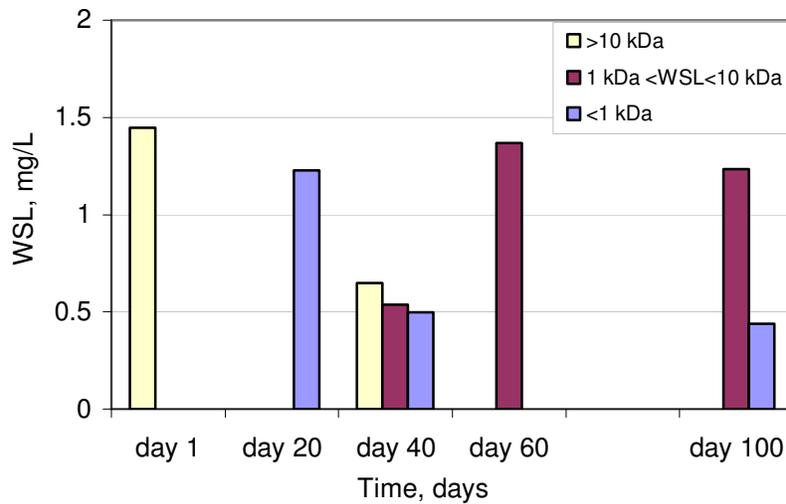
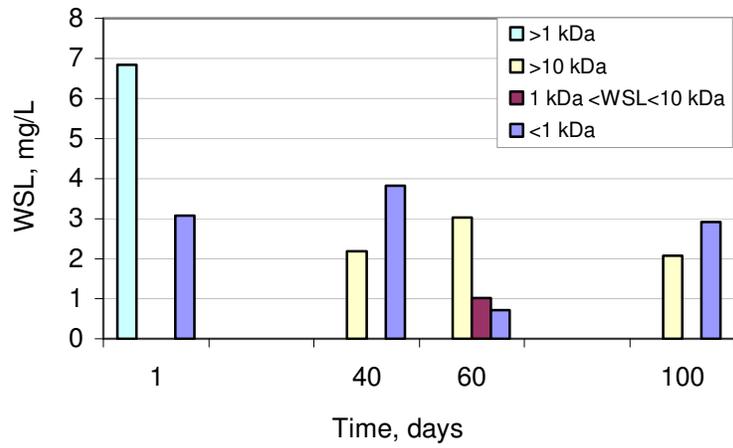
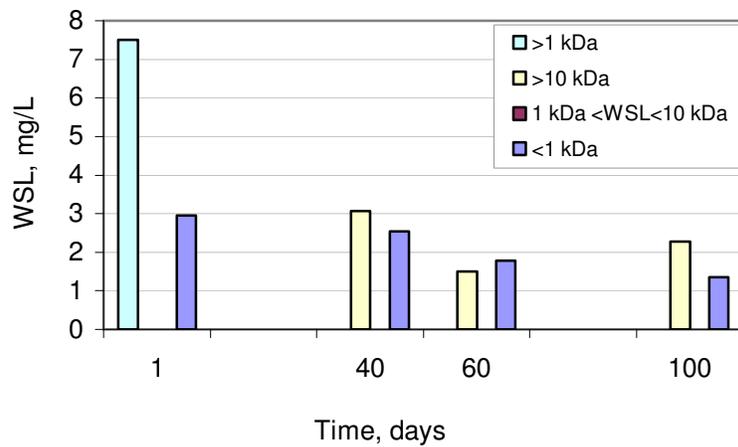


Figure 2.6: Water soluble lignin fractions size distribution for newspaper filled reactors with 23°C pretreatment and BESA amended seed.



(a)



(b)

Figure 2.7: Water soluble lignin fragments size distribution for newspaper reactors pretreated at 95°C with (a) and without BESA (b) amended cultures.

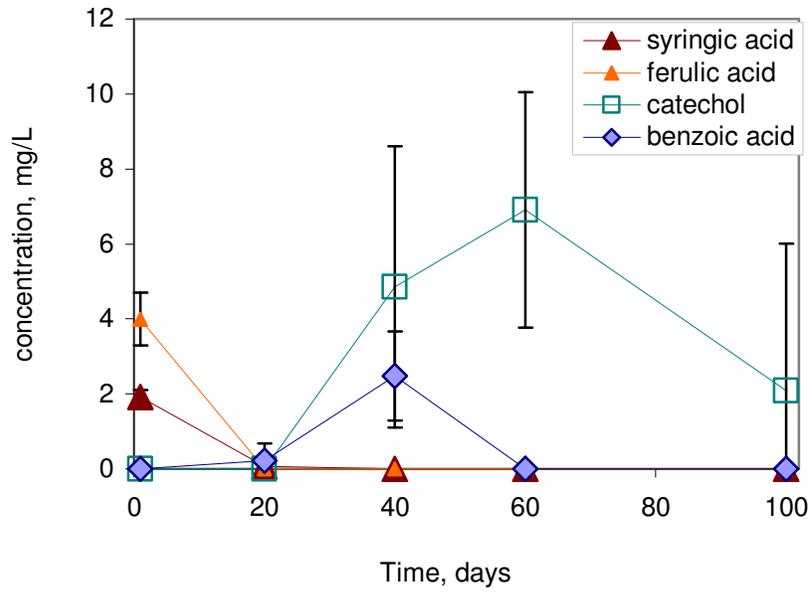
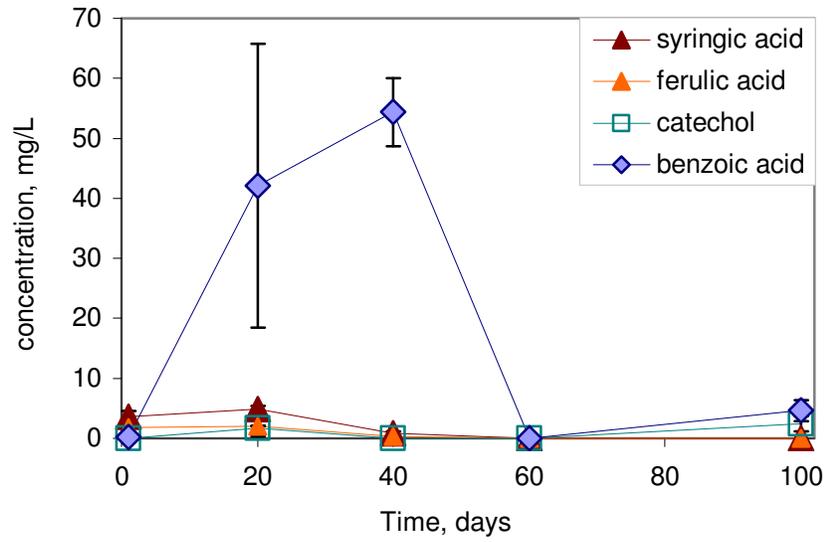
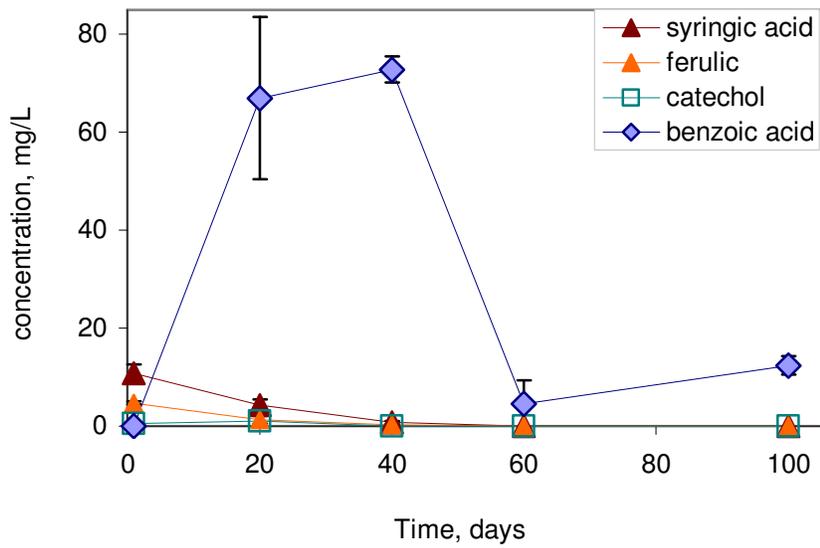


Figure 2.8: Lignin monomers release as a result of anaerobic biodegradation of synthetic lignin in 23°C pretreated reactors (error bars indicate 95% confidence over mean).



(a)



(b)

Figure 2.9: Lignin monomers release as a result of anaerobic biodegradation of synthetic lignin in 95°C pretreated reactors with partial inhibition of methanogens (a) and without (b) (error bars indicate 95% confidence over mean).

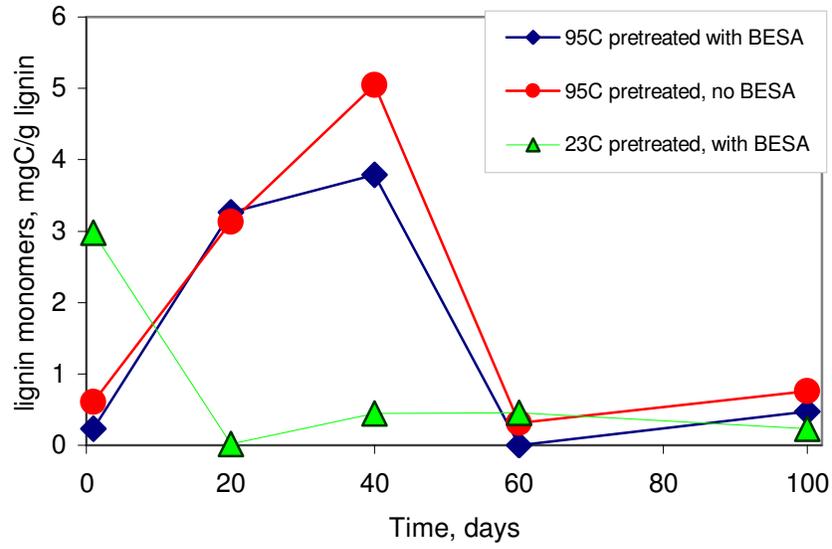


Figure 2.10: Total lignin monomers generation vs. time of incubation for synthetic lignin as a substrate.

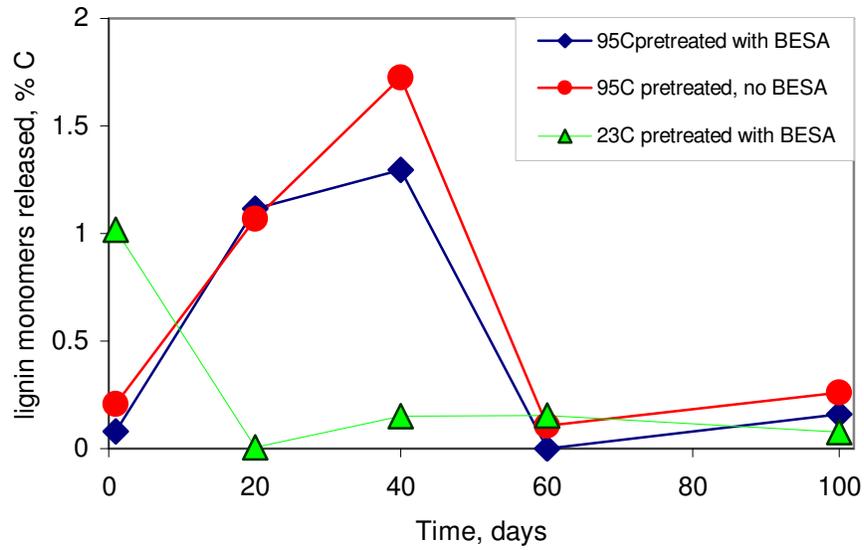


Figure 2.11: Percent of the total lignin monomers release from synthetic lignin as an organic carbon.

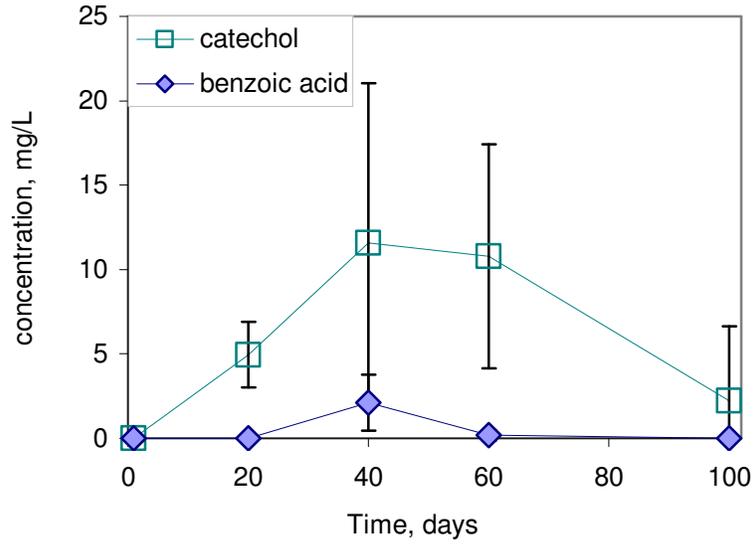
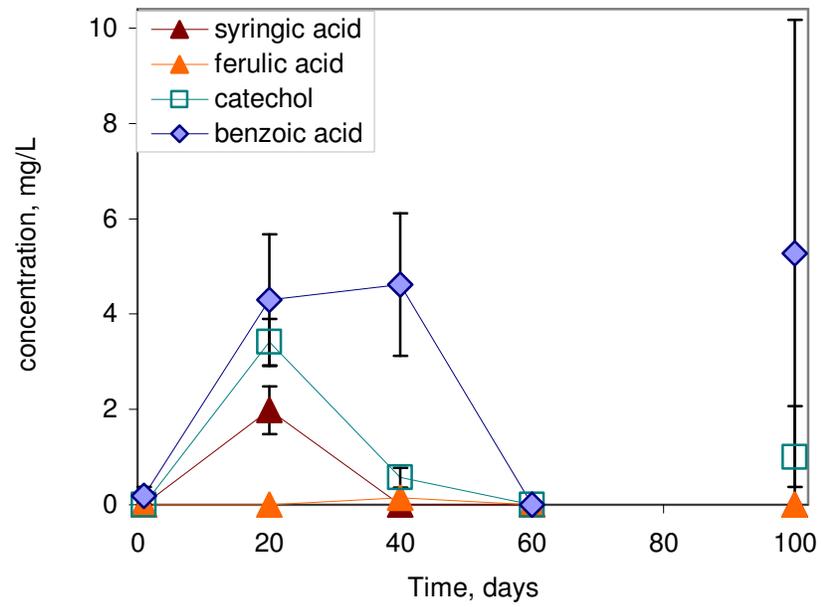
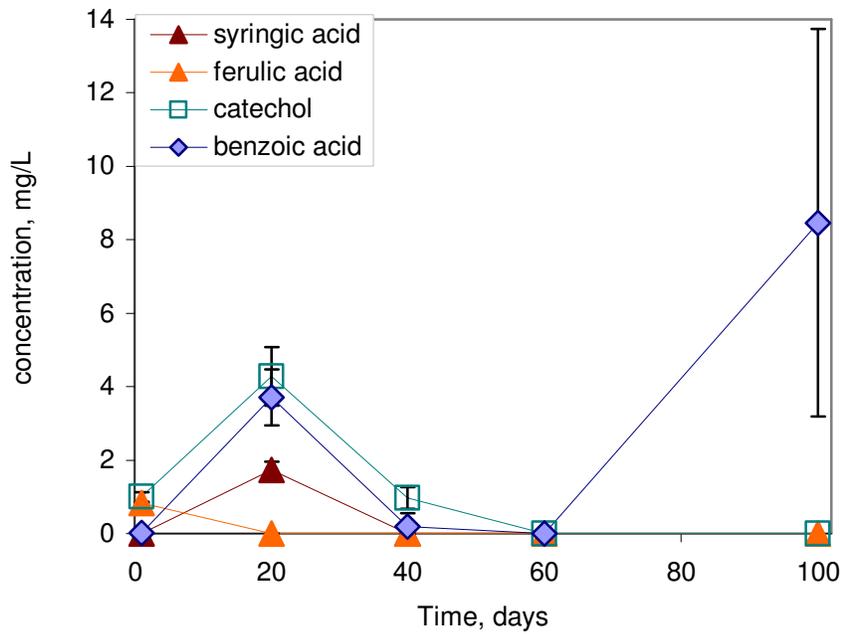


Figure 2.12: Lignin monomers release as a result of anaerobic biodegradation in 23°C pretreated newspaper reactors with BESA (error bars indicate 95% confidence over mean).

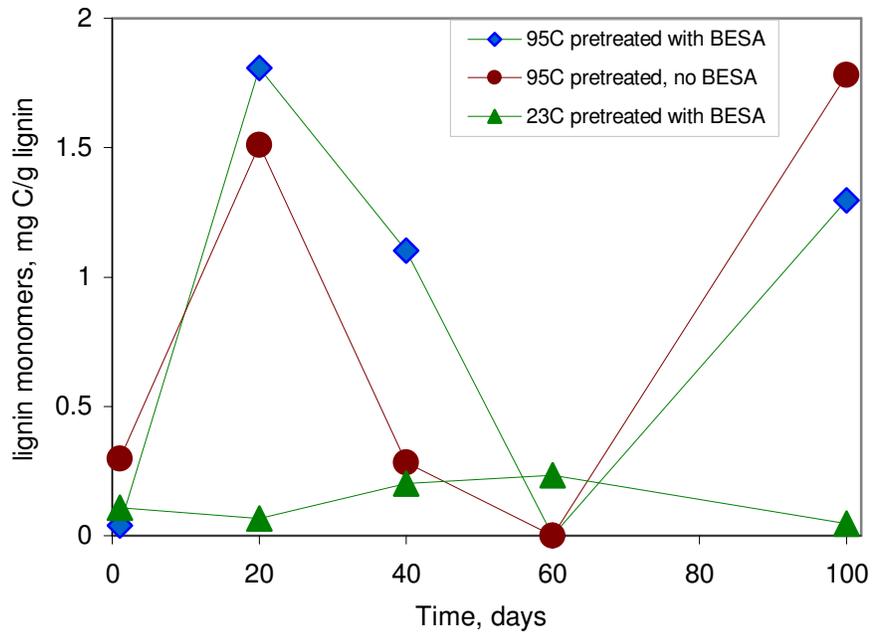


(a)

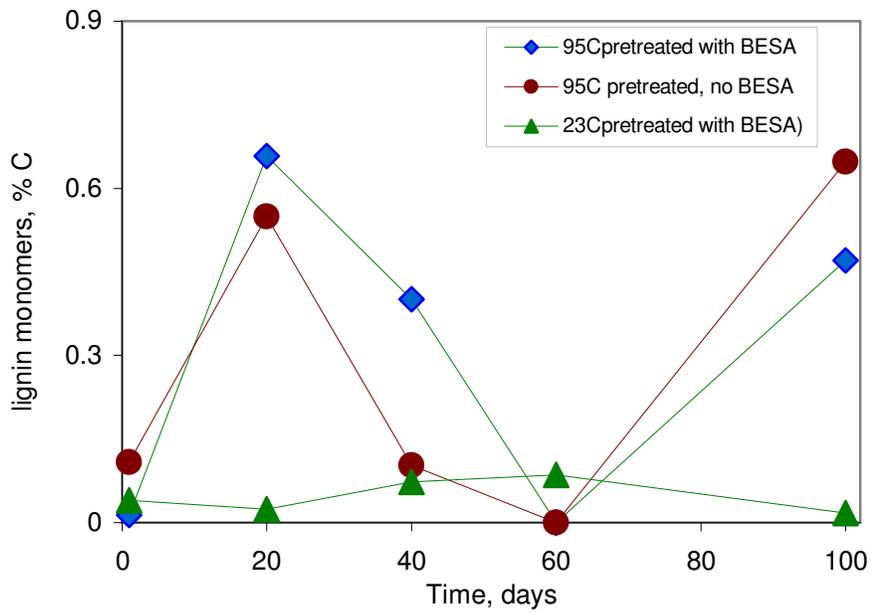


(b)

Figure 2.13: Lignin monomers release as a result of anaerobic biodegradation in 95°C pretreated newspaper reactors with BESA (a) and without BESA (b) (error bars indicate 95% confidence over mean).



(a)



(b)

Figure 2.14: Total lignin monomers generation vs. time of incubation for newspaper as a substrate: (a) expressed as mg organic carbon per g of lignin; (b) percent of monomers released as organic carbon per g of lignin.

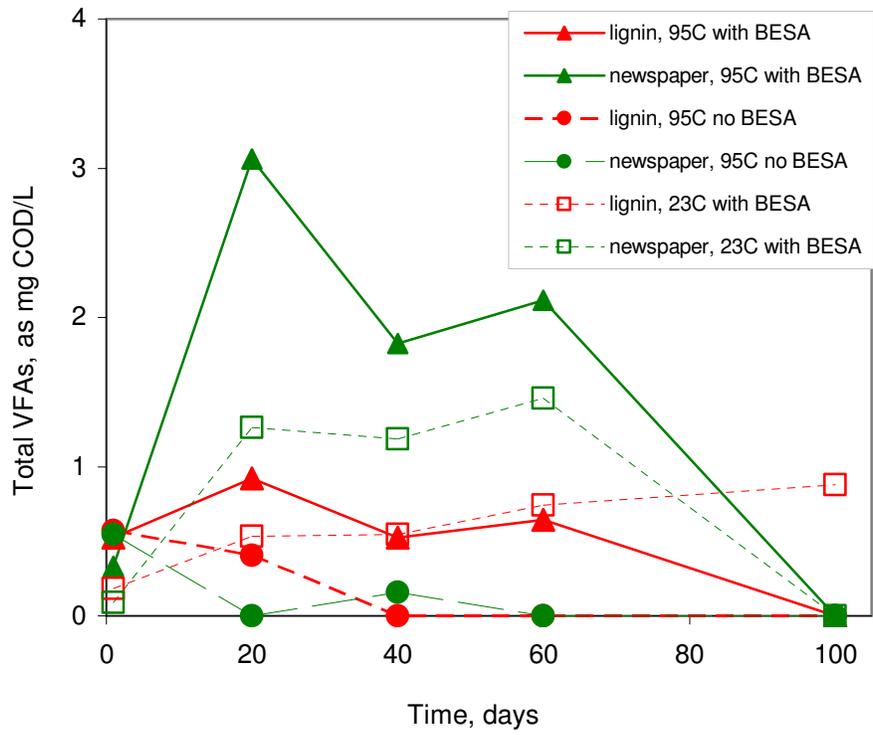
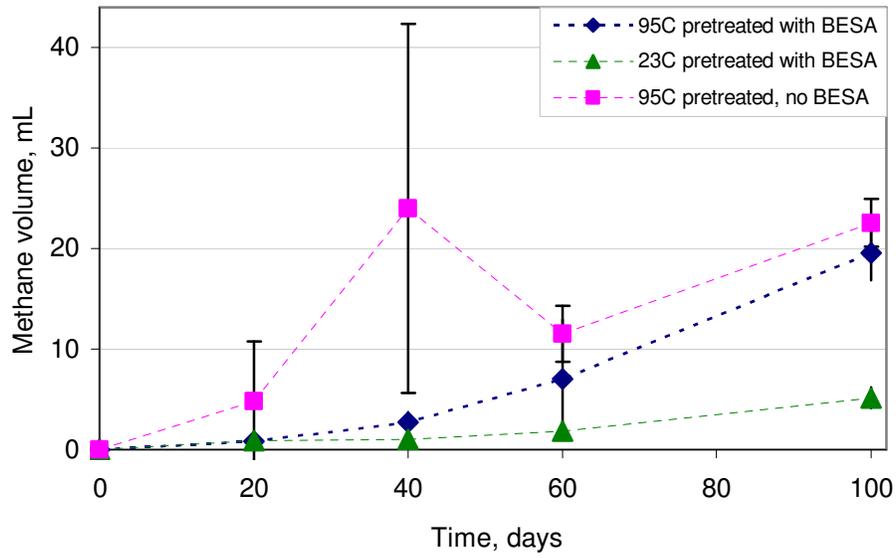
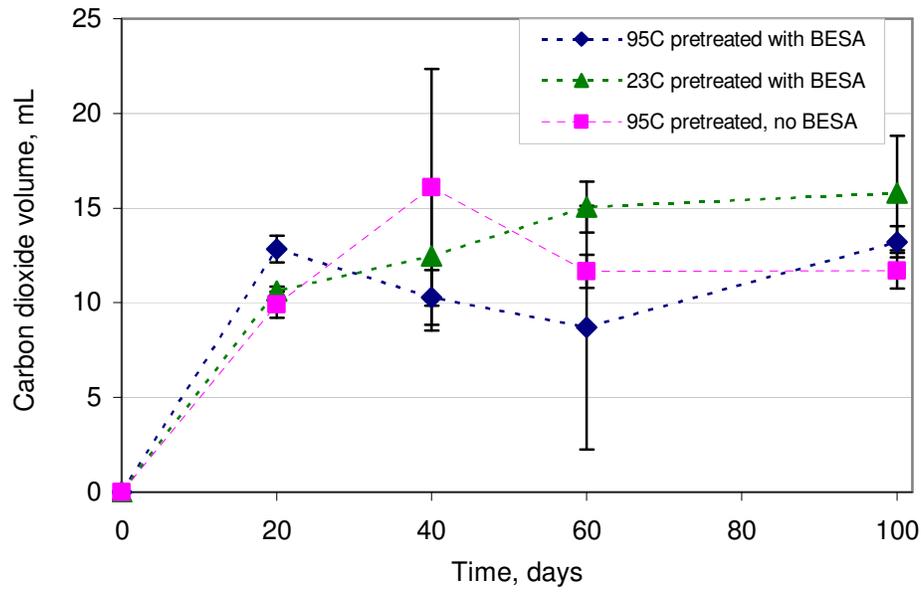


Figure 2.15: Total VFAs vs. time of incubation.



(a)



(b)

Figure 2.16: Biogas production vs. time of incubation of synthetic lignin: (a) methane; (b) carbon dioxide (error bars indicate 95% confidence over mean).

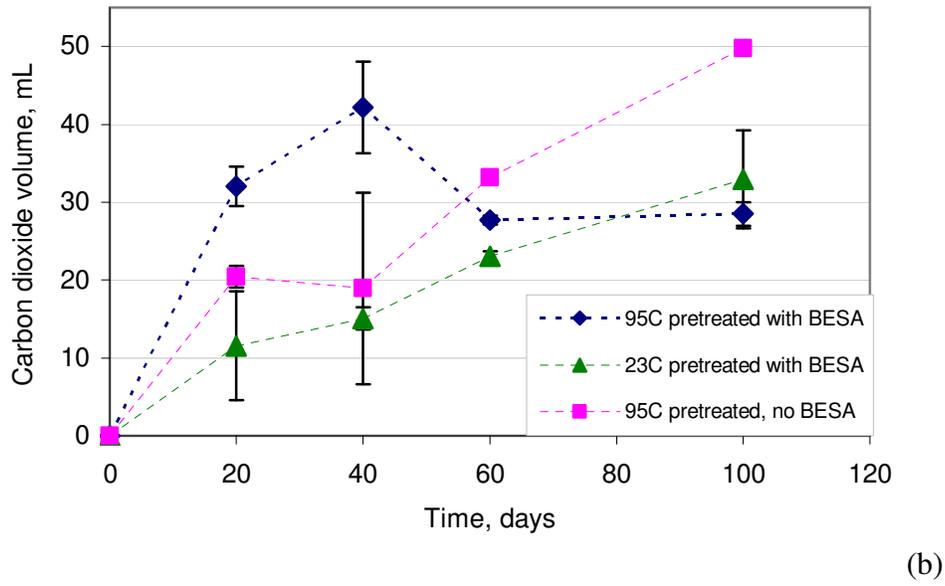
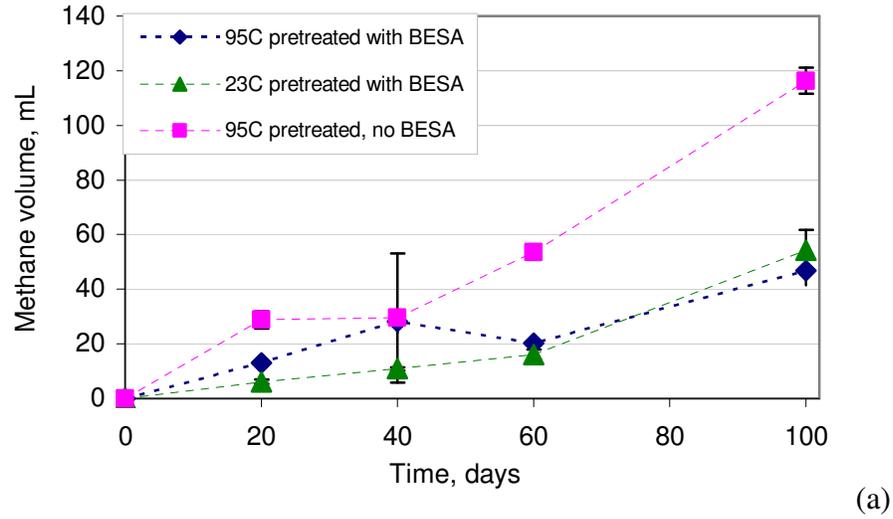


Figure 2.17: Biogas production vs. time of incubation of newspaper: (a) methane; (b) carbon dioxide (error bars indicate 95% confidence over mean).

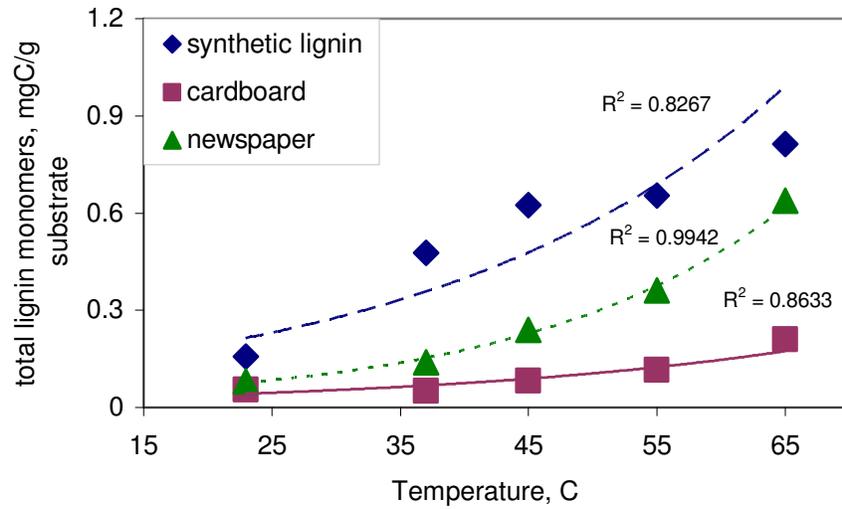


Figure 2.18: Lignin monomers release from synthetic lignin, and two types of paper monomers vs. temperature with exponential trends applied.

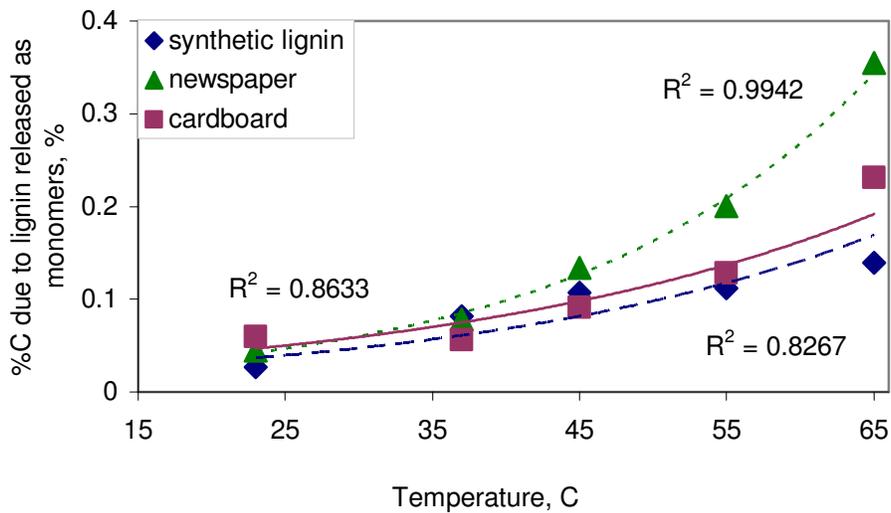


Figure 2.19: Percentage of carbon associated with lignin solubilization in form of lignin monomers vs. temperature of exposure for 48 hours.

## VITA

Olga Miroshnikova was born in 1977 in Moscow, Russia. She left Moscow public school № 142 in 1995. The same year she entered Moscow State Technological University “STANKIN” and graduated as a Bachelor in Technology, Equipment and Automation at Machine-Building Plants in 1999. She continued her study in “STANKIN” and in 2001 obtained Certified Engineer degree in Industrial Engineering: Environmental, Health, and Safety Aspects. In 2004 she started her M.S. degree at Virginia Polytechnic Institute and State University in USA and received her M.S. in the Environmental Engineering in September 2006.