Abstract

CHEN, YE. Effect of Aging on the Bioavailability of Toluene Sorbed to Municipal Solid Waste Components. (Under the direction of Dr. Morton A. Barlaz and Dr. Detlef R. U. Knappe).

The bioavailability of toluene sorbed to individual municipal solid waste (MSW) component [office paper, newsprint, model food and yard waste, high density polyethylene (HDPE) and poly(vinyl chloride)(PVC)] was evaluated. Effects of sorbent decomposition on toluene bioavailability were studied by evaluating biodegradable sorbents in both fresh and anaerobically decomposed form. To determine the effect of aging on toluene bioavailability, bioavailability tests were performed for MSW components that were in contact with toluene for 1, 30, and 180 days. At the termination of bioavailability test, sequential organic solvent extraction, alkali extraction, and combustion were used to determine the fate of toluene that was not available to bacteria. Lignocellulosic waste (fresh and degraded office paper, newsprint) was subjected to enzymatic hydrolysis and acid hydrolysis to determine the effect of individual biopolymers in paper on the sorption and bioavailability of toluene.

The bioavailability of toluene sorbed to MSW components was highest in HDPE, a rubbery polymer, followed by the biopolymers and finally PVC, a glassy polymer. Except for HDPE, aging significantly reduced the bioavailability of toluene sorbed to MSW components. Relative to the 1-day aging time, the bioavailability of toluene sorbed to biopolymers was reduced by 11-22% and 12-29% after 30 and 180 days of aging, respectively. For fresh and degraded office paper, the reduced bioavailability was a combination effect of aging and pH increase. Analysis of solid phase at the termination of bioavailability tests indicated that the remaining ^{14}C in sorbents was sequestered within and/or covalently bound to sorbent organic matter. Stronger association between sorbent organic matter and ^{14}C was observed during aging as less ^{14}C was recovered by organic solvent extraction and more ^{14}C was detected in the humic substances when aging time increased. Large molecular weight substances in the humic matter may form covalent binding with toluene and/or intermediates of toluene biodegradation. Humic acid had 3.7-24.3 times higher affinity for toluene than fulvic acid.

Enzymatic hydrolysis and bioavailability tests were conducted to identify the effect of individual biopolymers in paper on toluene sorption. Toluene release from cellulose and hemicellulose was not enhanced after enzyme addition, indicating that cellulose and hemicellulose exhibited limited sorptive capacity for toluene. Lignin controlled toluene sorption and bioavailability for both fresh and degraded newsprint. Bioavailability tests showed no significant difference between toluene sorbed to biopolymer composite (fresh and degraded newsprint) and their isolated lignins. However, the presence of lignin could explain only 54% of the sorption capacity of degraded office paper. Bioavailability of toluene sorbed to degraded office paper lignin showed a higher initial biodegradation rate and mineralization extent than toluene sorbed to degraded office paper composite. Crude protein and lipophilic extractives were likely to contribute to the higher sorptive capacity of biopolymer composite. Lipophilic extractives provided highly hydrophobic environment for toluene uptake and caused the declined toluene bioavailability.

EFFECT OF AGING ON THE BIOAVAILABILITY OF TOLUENE SORBED TO MUNICIPAL SOLID WASTE COMPONENTS

by

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李白

谁纵千救眼三将闲事十银赵
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首惭赫郸气岳觞剑藏里沓钩
太世大先素倒劝膝身不如霜
玄上梁震霓为侯前与留流雪
经英城惊生轻赢横名行星明

Biography

Ye Chen received her B.S. of Environmental Engineering from Beijing Polytechnic University, Beijing, P. R. China, in 1996 and her M.S. of Environmental Engineering from the same university in 1999. In the Fall of 1999, she came to the United States to start her Ph.D. studies in the Department of Civil Engineering at North Carolina State University. She received her Ph.D. degree in Aug. 2003.

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Chapter 1

Introduction

Despite increases in recycling and incineration, landfills remain the major waste disposal option in the United States. In 1994, approximately 61% of the municipal solid waste generated was disposed of by burial in a sanitary landfill (U.S. EPA. 1996). In the year 2000, there are around 1960 operating municipal waste landfills in the US in addition to thousands of closed sites (U.S.EPA, 2002). Unlined landfills have been shown to result in groundwater contamination (Robinson and Barr 1999; Kettunen *et al*. 1999; Cozzarelli *et al*. 2000). Approximately 15% of the sites on the National Priority List of Superfund are municipal landfills that accepted hazardous waste (U.S. EPA. 2003). Alkylbenzenes, ketones and chlorinated aliphatic hydrocarbons are typical contaminants in ground water (Christenssen *et al*. 1994). Because of its water solubility and acute toxicity and genotoxicity, toluene was classified as priority pollutants by the U.S. EPA.

Landfills are physically and chemically complex ecosystems. Upon being deposited into landfills, organic compounds are subjected to a series of physical, chemical and biological processes. Active subsurface microflora is able to degrade many organic compounds, which leads towards remediation of these compounds from landfill environment. However, sorption of chemical to solids is one mechanism that prevents the chemicals from being degraded by microorganisms (Scow and Johnson 1997). Sorption also reduces the mobility of contaminants. Organic compounds may be immobilized through either diffusion into municipal solid waste (MSW), which is a reservoir of organic matter, or by degradation products forming covalent bonds with humic substances. Sorbed compounds are more resistant to microbial degradation as indicated in previous research (Pignatello *et al*. 1993; Lamoureux and Brownawell 1999; Alexander 2000). In the case of hydrophobic organic contaminants (HOCs), biodegradation and sorption can be viewed as competitive processes. If the biodegradation rate is rapid, the compound will disappear before sufficient time elapses for appreciable sorption. On the contrary,

the slower the disappearance of the compound, the more time is available for sorption and loss in bioavailability to occur (Nam and Alexander 2001). Bioavailability of organic compounds is determined by the characteristics of the sorbent (Luthy *et al*. 1997), and the sorbate (Brusseau and Rao 1991), the physiological traits of microorganisms (Scow and Johnson 1997), and environmental factors (Rijnaarts *et al*. 1990).

To date, little information is available on the bioavailability of HOCs sorbed to MSW. Most studies concerning bioavailability of sorbed organics have been conducted in soils and sediments. MSW differs from soils/sediments with respect to contaminant fate and bioavailability in that (1) compared to soils/sediments; MSW has a higher organic carbon content (Eleazer *et al*. 1997). Glassy organic matter is hypothesized as a major factor leading to slow diffusion and reduced bioavailability of HOCs (Pignatello and Xing 1996); (2) biodegradation of lignocellulose and microbial synthesis lead to the formation of humic substances (Shevchenko and Bailey 1996). Previous investigations revealed humic substances were responsible for both sequestration (White *et al*. 1999) and irreversible binding of HOCs to the solid phase (Bollag *et al*. 1998) and (3) many studies show bioavailability is reduced by aging, a prolonged contact of HOCs to soils/sediments (Hatzinger and Alexander 1995; Nam and Alexander 1998), similar effect is expected in landfills, where long contact time can lead to the diffusion of HOCs into hard/condensed organic matter or micropores in sorbents.

Investigating the bioavailability of HOCs sorbed to MSW is of considerable importance in attempts to predict the fate and transport of these compounds and the extent of possible bioremediation in landfill. The result will also provide useful information in developing models to predict contaminant fate in landfills. Such models are essential to project contaminant concentrations in leachate, which can be used to assess the leachate contamination risk to groundwater. Moreover, since aging leads to a loss in acute toxicity to organisms, the ability to predict the extent of sequestration is of great importance in risk analysis.

To develop a more fundamental understanding of the bioavailability of HOCs sorbed to MSW, the research was designed to meet the following objectives:

- Investigate the impacts of aging and sorbent decomposition on bioavailability.
- Compare rates of desorption and biodegradation; evaluate whether contaminant desorption limits bioavailability.
- Investigate the effect of individual lignocellulosic materials (cellulose, hemicellulose, and lignin) as well as their decomposition on toluene bioavailability.

Chapter 2

Literature Review

This literature review is composed of four parts: characterization of MSW; bioavailability; factors controlling bioavailability; and aerobic degradation of toluene. The first part is a summary of municipal solid waste components. Considering the importance of organic matter in bioavailability, the individual components of organic matter (cellulose, hemicellulose, lignin etc.) in solid waste as well as their biodegradation and effect on sorption, humification are reviewed. In the following part, a brief definition of bioavailability and summary of published research involving bioavailability of organic compounds are provided. Literature on soils and sediments is emphasized because there is no published information on bioavailability in the landfill ecosystem. In the third part, mechanisms that cause reduced bioavailability are explained. Specifically, this part will focus on the factors controlling bioavailability such as aging and microorganisms. The following part includes information about aerobic toluene biodegradation; different biodegradation pathways and toluene oxidizing bacteria.

2.1 Characterization of Municipal Solid Waste

Municipal solid waste contains organic materials such as paper, food and yard waste and plastics. To be specific, MSW can be divided into six major chemical compound classes: non-cellulosic carbohydrates (hemicellulose, starch, and mono- and oligosaccharides), cellulose, proteins, lipids, lignin, and plastics (Pichler and Kögel-Knabner 2000). Paper and paperboard products make up the largest component of MSW. Of the total 229.9 millions of tons of MSW generated in the U.S. in 1999, 38.1% were paper products. Although 41.9% was recovered by recycling, there were still 50.8 million tons of paper that were deposited in landfills (US EPA 1999). Newsprint is produced from mechanical pulp with some chemical wood pulp. Only 7% of the raw materials are lost during the production of newsprint. Therefore, most compounds of the wood, lignin, cellulose, hemicellulose are present in newsprint. Office paper is made from a

chemical pulp of high purity. Most of the lignin is removed during chemical treatment, leaving cellulose as the major component. The percentage of the cellulose varies depending on specific chemicals used (Calkin 1957). Food waste comprised 12.1% of MSW; the major organic components of food waste are carbohydrate, protein and lipids (US EPA 1999).

2.1.1 Cellulose and Hemicellulose

Cellulose, a linear polymer made of glucose subunits linked by β-1, 4 glycosidic bonds, is the most abundant biopolymer on earth (Senior 1990). Most native celluloses are composed of two different forms. The parallel oriented chains form highly ordered crystalline domains. The crystalline domain is interspersed by more disordered, amorphous regions. The native crystalline form of cellulose has a structure designated as type I, which can be converted into type II by alkali treatment. Depending on origin and pretreatment, the degree of crystallinity of cellulose can vary from 0% to 100% (Béguin and Aubert 1994). Evans *et al*. (1995) studied the crystallinity change during Kraft pulping process using X-ray diffraction, infrared (IR) spectroscopy and NMR spectroscopy. All three methods indicated that the degree of crystallinity of the cellulose increased as Kraft pulping proceeded due to preferential removal of the less ordered carbohydrates. Hunt *et al*. (2001) also reported cellulose fibril distortions were partially relaxed by the pulping process, which led to significant cellulose crystallinity increase.

Water decreases the glass transition temperature (T_g) of dry cellulose from 225 °C to -45 °C (Leboeuf *et al*. 2000). In aqueous solution, HOC sorption to cellulose should therefore be dominated by partitioning mechanism. Pure cellulose has a fairly low sorption capacity for organic compounds, with 1.0 mg/g for benzene and 1.4 mg/g for tetrachloride phenol (Rutherford *et al*. 1992). The low uptake of cellulose is determined by its high organic polarity (O+N)/C (Rutherford *et al*. 1992) and lack of aromaticity (Xing *et al*. 1994).

Cellulolytic microorganisms are found among diverse taxonomic groups. They usually occur in mixed populations comprising cellulolytic and non-cellulolytic species, which often interact synergistically (Béguin and Aubert 1994). Cellulose is highly insoluble in water. Cellulolytic

microorganisms can hydrolyze the 1,4-glycosidic bonds of cellulose with cellulase, which can convert the complex cellulose to smaller cellobiose molecules (Pelczar Jr. and Reid 1958). Cellulases form a multicomponent enzyme system; with endoglucanases (EGs) that hydrolyse cellulose chains randomly, cellobiohydrolases (CBHs) that hydrolyse cellobiose from the polymer ends and cellobiases that hydrolyse cellobiose to glucose (Cavaco-Paulo 1998). The individual component and their mode of action are summarized in Table 2.1 (Bhat and Bhat 1997). Amorphous cellulose is preferentially hydrolyzed while crystalline cellulose is more resistant to hydrolysis (Senior 1990). In both aerobic and anaerobic conditions, cellulose is substantially degraded by fungi and bacteria (Eleazer *et al*. 1997; Pichler and Kögel-Knabner 2000). In most natural environments, cellulose cannot be completely mineralized due to the protective effect of lignin, soil minerals and humus polymers. Cellulose can form chemical or physical linkages to these constituents to persist for a sufficient length of time to participate directly in humus formation (Bollag *et al*. 1998).

Enzymatic hydrolysis was used recently to investigate of the residual lignin and lignin-cellulose bonds in pulps. Karlsson *et al*. (2001) used cellulases and hemicelluases to study lignin-cellulose as well as lignin-hemicellulose bonds in Kraft pulps. It was found 40% of pine Kraft pulp was degraded by the cellulase treatment. The remaining cellulose was still of high molecular weight, which indicated a considerable portion of the residual lignin in the pine Kraft pulp was bonded to cellulose. The authors also reported the simultaneous removal of lignin and cellulose by the cellulase treatment. This result is similar to that of Hortling *et al*. (1990), who concluded that the 20-60% residual lignin dissolved during the enzymatic hydrolysis of pulps with a kappa number (most common parameter for determination of lignin content) below 58.

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TABLE 2.1 Components of Aerobic Fungal Cellulases

Enzyme	Synonym	EC code	Mode of Action
endo-1,4-β-D-glucanase	1,4-β-D-glucan glucanohydrolase	EC 3.2.1.4	-G-G-G-G-G ↑ ↑ Cleaves linkages at random
exo-1,4-β-D-glucanase	1,4-β-D-glucan cellobiohydrolase	EC 3.2.1.91	-G-G-G-G-G- ↑ Releases cellobiose either from reducing or non-reducing end
		EC 3.2.1.74	-G-G-G-G-G- ↑ Releases glucose from non-reducing end
β-glucosidase	cellobiose β-D-glucoside glucohydrolase	EC 3.2.1.21	G-G G-G G-G ↑ ↑ Convert cellobiose to glucose

Hemicelluloses contain xylan, mannan, galactan and arabinan as the main heteropolymers (Dekker and Richards 1976). The principal monomers present in most hemicelluloses are D-xylose, D-mannose, D-galactose, and L-arabinose (Wenzl 1970). For native hemicelluloses, the most probable T_g is around 180 °C, and 30% moisture can lower the T_g to room temperature (Back and Salmen 1982). Because of the much greater complexity of sugars and linkages in hemicelluloses, many more enzymes are involved in the complete hydrolysis of the backbone and the branches (Deobald and Crawford 2000). Because xylan is a common hemicellulose backbone constituent, much of the research on hemicellulases has focused on xylanases. The xylanolytic enzyme system is composed of β-1,4-endoxylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, and phenolic acid esterase. All these enzymes act cooperatively to convert xylan into its constituent sugars (Beg *et al*. 2001). Sugars produced can be readily detected colorimetrically (Sharrock 1988) using method proposed by Miller *et al*. (1960), Lever (1973), Mullings and Rarish (1984).

2.1.2 Lignin

Lignin is, after cellulose and hemicellulose, the third most abundant biopolymer on earth. It is present in the primary cell wall, which provides mechanical strength. The polymer is composed of aromatic alcohols, particularly three p-hydroxycinnamyl alcohols. Lignin plays an important

role in humification processes. One of the widely accepted theories concerning organic matter humification in soil is that lignin and its degradation products such as phenols, quinones and more complex compounds are the main precursors in the formation of humic substances; their polymerization and condensation with N-compounds such as protein, amino acids, nucleic acids is brought about by the soil microorganisms (Stevenson 1994; Sánchez-Monedero *et al*. 1999). Lignin is a glassy polymer with a glass transition temperature of 70 °C (LeBoeuf *et al*. 2000).

White-rot fungi are responsible for most of the lignin decomposition in nature and lignin degradation by white-rot fungi is faster than by any other organism (Tuomela *et al*. 2000). There are many genera of actinomycetes and eubacteria that can degrade extracted lignin and dehydrogenation polymer (synthetic lignin). Many bacterial strains, especially actinomycetes, can solubilize and modify the lignin structure extensively, but their ability to mineralize lignin is limited (Buswell and Odier 1987). Aerobic microorganisms are the primary lignin degraders in most environments. Anaerobic degradation of lignin is either not observed (Micales and Skog 1997; Pichler and Kögel-Knabner 2000; Odier and Monties 1983) or happens at very low speed under specified conditions. It was found that anaerobic rumen microorganisms are capable of degrading plant fiber cell wall (Kuhad *et al*. 1997). Colberg (1988) investigated the anaerobic microbial degradation of lignin compounds and concluded that the intermediate metabolic products called oligolignols may be partially degraded to CO_2 and CH_4 by anaerobic microorganisms. Benner and Hodson (1985) found elevated temperature of 55° C could enhance the anaerobic degradation of lignin.

2.1.3 Poly (vinyl chloride) (PVC) and High Density Polyethylene (HDPE)

PVC is composed of repeating vinyl chloride monomers ($-CH_2-CH-Cl$). In its unmodified form, PVC is a rigid polymer. Plasticizers are organic additives added to PVC compounds in order to improve their processing properties and make flexible products for a wide variety of purposes (Mersiowsky *et al*. 2001). PVC is a stable polymer in landfill environment. No biological or abiotic mechanisms for a depolymerisation process are known. Temperatures usually encountered in the landfill are in the range of 18-55°C, which are substantially lower than

temperatures required for thermal destruction (Mersiowsky *et al*. 2001).

Plasticizers are important in determining the glass transition temperature of PVC and consequently its sorptive behavior. Rigid PVC undergoes glass transition at temperatures between 60 and 80 °C, whereas for flexible PVC compounds this temperature range is reduced to around 0 °C at an average plasticizer content of approximately 30% (Mersiowsky *et al*. 2001). Significant plasticizer loss can occur in landfill due to microbial transformation during the methanogenic landfill stage (Ejlertsson *et al*. 1997; Mersiowsky *et al*. 2001), which indicates that PVC containing plasticizers may be transformed from a rubbery to a glassy state in landfill environment.

Polyethylene consists of repeating ethylene (CH_2=CH_2) monomers. The glass transition temperature of polyethylene is –68 °C (Brandup *et al*. 1989). HDPE contains interstitial spaces between the polymer segments through which small molecules can diffuse.

2.1.4 Protein and Lipid

Proteins are one of the major constituents of living organisms. Proteins are made up of one or more polypeptide chains, each consisting of many α-amino acid residues covalently linked by peptide bonds. The naturally occurring proteins are too large to enter the bacterial cell. The decomposition of protein occurs in two stages:

$$\text{Protein} + H_2O \xrightarrow{\text{Enzymes}} \text{polypeptides}$$

$$\text{Polypeptides} + H_2O \xrightarrow{\text{Enzymes}} \text{mixture of individual amino acids}$$

The amino acids taken into the cell may be used for the formation of proteins or subjected to further degradation to ammonia and CO_2. Analysis of organic matter of municipal solid waste showed no significant change in protein content during aerobic and anaerobic treatment (Pichler and Kögel-Knabner 2000). One mechanism attributed to the persistence of protein or peptide is the microbial resynthesis of protein. Proteins may be preserved by encapsulation into refractory

cell wall polymer of microorganisms, e.g., lipids, and become poorly degradable (Dinel *et al*. 1996; Lichtfouse *et al.*1996) and may be incorporated into humic substances (Sánchez-Monedero *et al*. 1999).

Lipids include a variety of fat-like substances. Many bacteria are capable of decomposing fats through lipases. Lipases cause a hydrolytic breakdown through addition of water; the lipid molecule is split into glycerol and its respective fatty acid. González-Vila *et al*. (1995) studied the progressive transformation of lipids in landfills. Samples were taken from different depths in the landfill. The depth of the samples represented the disposal time. The amount of Sohxlet-extractable lipids showed irregular behaviors with depth, which might be due to the great heterogeneity of the samples and microbial resynthesis. Microbially synthesized long-chain lipids are resistant to attack by microorganisms (Dinel *et al*. 1996).

2.2 Bioavailability

Bioavailability represents the accessibility of a chemical for bioaccumulation, where bioaccumulation is the accumulation of a contaminant via all routes available to the organism (Alexander 2000). Sorption processes have been shown to influence the bioavailability of various organic chemicals. As a result of sorption both increases and decreases in biodegradation rate are possible. Sorption often has been found to stimulate biodegradation when the compound to be degraded or its metabolites are toxic to the microorganisms. In this case sorption reduces the toxic chemical concentration. Ehrhardt and Rehm (1985) found cells of *Pseudomonas* and *Candida* can not degrade phenol at concentration higher than 1.5 g/L. The presence of activated carbon enhanced the biodegradation by quickly reducing the toxic phenol concentration and set low quantities of adsorbed phenol free for gradual biodegradation. More often, sorption has been reported to decrease bioavailability (White *et al*. 1999; Steinberg, *et al*. 1987; Hatzinger and Alexander 1997), although there are inconsistencies regarding the ability of microorganism to degrade sorbed pollutants (Tang *et al*. 1998, Laor *et al*. 1996). Most studies support the concept that contaminants that partitioned into natural organic matter (Calvillo and Alexander 1996; Chung *et al*. 1993; Crocker *et al*. 1995; Harms and Zehnder 1995) or diffused into micropores

(Nam and Alexander 1998; Hatzinger and Alexander 1997, Löser *et al*. 1999) are not directly available for degradation. Technologies such as addition of nutrients, electron acceptor and bacteria are unlikely to stimulate the degradation of pollutants that are sequestered (Hatzinger and Alexander 1997). Only when organic compounds desorb out of sorbents to a site where they are freely available to microorganisms can they be degraded. Desorption of organic compounds out of organic matter can be very slow due to the mechanisms described below. In most cases, the lower desorption rate limits degradation rate and bioavailability is limited by desorption.

2.3 Factors Controlling Bioavailability

2.3.1 Aging Effect

Sorption and consequently the loss of bioavailability is a time-dependent process. Numerous studies showed contaminant bioavailability to microorganisms decreases with time up to a time beyond which a further decline is no longer detectable (Table 2.2). Sorption has been observed to occur in two stages: a rapid step involving association with labile domains near the particle surface, followed by a slower step (Pignatello and Xing 1996; Johnson *et al*. 1999; Brusseau *et al*. 1991). Karickhoff (1984) showed that sediments continue to sorb hydrophobic compounds from water indefinitely at a slow rate after the initial rapid uptake. Continued slow sorption can be attributed to HOCs diffusing into remote micropores or hard/condensed organic matter (Alexander 2000). During aging, more molecules move into sites from which desorption is retarded; this process is responsible for the reduced bioavailability as aging time increases. In the following section, the mechanisms behind the aging phenomenon are explained.

TABLE 2.2 Compounds Shown to Become Less Bioavailable as a Result of Aging

Compounds	Sorbent	Aging period	% Non-bioavailable	Reference
4-nitrophenol	Soil (lima loam)	103 d	20.5	Hatzinger and Alexander 1997
Naphthalene	Soil	3 years	25	Guerin and Boyd 1997
Phenanthrene	Soil	103 d	31	White *et al*. 1999
Phenanthrene	NaOH extracted soil	103 d	29.5	White *et al*. 1999

Sorption to Hard/Glassy Organic Carbon

Organic matter consists of humic substances and partially decomposed biomass. When present above trace levels, organic matter is the predominant sorbent of hydrophobic organic compounds (Xing and Pignatello 1997). The OM is viewed as a tangle of macromolecules that offers an organo-lipophilic phase. Evidence supporting sorption by OM was provided by Dec (1997), who used ^{13}NMR to study residues of fungicide cyprodinil. The analysis of fulvic acid and humin revealed they contained mainly unmodified cyprodinil molecules, which was apparently sequestered in soil by physical forces other than covalent binding.

Organic matter among different soils or within the same soil differ in polarity, elemental composition, aromaticity, condensation and degree of diagenetic evolution (Weber *et al*. 1992; McGinley *et al*. 1993; Young and Weber 1995). Therefore, observations of sorptive behavior vary. Organic matter is further modeled as a combination of rubbery (soft carbon) and glassy (hard carbon) polymers (Pignatello and Xing 1996; Weber and Huang 1996). The rubbery (soft carbon) phase is oxidized, amorphous, expanded, soft, loosely knit organic matter domain with flexible, swollen pores (Huang and Weber 1997). Such highly amorphous organic matter domains act as liquid-like partitioning phases for hydrophobic solutes. Consequently isotherms are linear and no hysteresis is observed. HOC sorption to and desorption from the rubbery (soft carbon) phase is faster than for the glassy (hard carbon) phase. The glassy phase is composed of condensed, relatively reduced, less polar, hard organic matter with relatively well-defined, rigid mesopores and micropores (Xing and Pignatello 1997). Under aqueous conditions, water molecules are unlikely to penetrate and expand such hydrophobic matrices (Huang and Weber 1997). Sorption of hydrophobic molecules into glassy phases can be explained by dual model sorption (Xing and Pignatello 1996). Dual-mode sorption is the sum of normal linear partitioning taking place in the bulk of the polymer and a hole-filling mechanism in which the incoming molecules undergo Langmuir-like adsorption in voids internal to the polymer matrix. Glassy OM can be converted to rubbery OM by increasing the temperature above glass transition point or by softening it by organic solvent. Diffusion into organic matter is often termed as sequestration. Unlike bound compounds, those that are sequestered can be recovered from soil by exhaustive extraction with organic solvents, but their bioavailability and toxicity gradually diminish as a

result of aging (Dec *et al*. 1997).

Sorption into glassy/hard organic matter is a possible rate limiting step in desorption because (1) the pressures associated with sorption are significantly greater than those involved in desorption. Hydrophobic solute molecules may hysteretically adsorb or condense within such pores; (2) diffusion in or out of tightly cross-linked and condensed OM is usually more highly activated and can be very slow.

Weber and Huang (1996) studied the change of phase distribution relationships with time under nonequilibrium conditions. They found sequence of accessibility of pollutant to each component and the associated dominant mechanism in soil is time dependent. Soft or rubbery organic matter is readily accessed by HOC molecules and associated sorption rates are relatively fast. The mechanism of sorption to this domain is one of partitioning. Once the readily accessible domain site reaches apparent equilibrium, the condensed organic matter domain is accessed and the overall sorption process is dominated by the behavior of this domain. Aged samples, for which sorbent-sorbate contact time may be months or years, can be enriched in the slow fraction owing to the slow diffusion into condensed organic matter.

Diffusion through Hydrophobic Micropores

Another mechanism proposed to explain the slow desorption and sequestration is retarded diffusion through and along micropores (Steinberg *et al*. 1987; Brusseau *et al*. 1991) and micropore geometries giving rise to different mechanisms of desorption (Werth and Reinhard 1997). To differentiate the retarded diffusion through organic matter and micropores, Cornellissen *et al*. (1998) tested desorption from model sorbents without organic matter and sediment whose organic matter had been completely removed. Significant slow and very slow desorption fractions were observed for these materials. This result indicated that the presence of pores with hydrophobic walls resulted in strong desorption retardation. Similar results were reported by Farrell *et al*. (1999), who studied the desorption rates of chloroform, TCE, and PCE from silica gel and concluded pores less than 2 nm in diameter were responsible for slow desorption.

There is inconsistency in the pore classification between IUPAC (International Union of Pure and Applied Chemistry) and SSSA (Soil Science Society of American). Table 2.3 summarizes the classification used by IUPAC (1972) and SSSA (1997), and IUPAC definition will be used throughout this research. Release of contaminants sorbed in micropores may be hindered by high adsorption energies. Micropore desorption has been reported to be an activated process. Everett and Powl (1976) calculated the adsorption energy might be up to a factor of 3.5 times greater than in micropores compared to that on an open surface. Higher adsorption energies lead to reduced diffusive transport rates (Farrell and Reinhard 1994a). Besides increased adsorption, micropores also represent potential sites for steric entrapment of sorbing molecules. The molecules might be effectively trapped and only become bioavailable through desorption and subsequent diffusion through a tortuous pathway. Movement through that tortuous path would be exceedingly slow because of the steric hindrance, reduced advection of water and turtuosity of pore network. As the pore size decreases, the ratio of pore surface area to pore volume increases. The result is that a given sorbate molecule spends relatively more time adsorbed on the surface than in solution for smaller pores compared to larger pores. Transport rates are reduced because the relative slowness of surface diffusion compared to aqueous diffusion (de Boer 1968). Nam and Alexander (1998) conducted bioavailability test on model solids: glass beads without pores, silica beads with nanopores, polystyrene beads with hydrophobic surfaces but no pores, diatomite beads with comparatively large pores, and polystyrene beads with hydrophobic surfaces and nanopores. The result showed neither hydrophobicity nor surface area alone rendered phenanthrene unavailable to bacteria. However, bioavailability of a hydrophobic compound such as phenanthrene can be markedly reduced by particles bearing nanopores having hydrophobic surfaces. In contrast, nanopores with hydrophilic surfaces or particles without nanopores did not reduce bioavailability.

TABLE 2.3 Classification of Pore Size

	Macropores	Mesopores	Micropores
IUPAC (1972)	> 50 nm	2-50 nm	< 2 nm
SSSA (1997)	>75 μm	30-75 μm	5-30 μm

Humification

The classical theory suggests that humic substances are formed through modification of lignin. Another theory in agreement with this suggests that humic acids are derived from quinones, which in turn are formed from polyphenols or lignin decomposition products (Stevenson 1994). Humification is composed of two stages: The first stage involves degradative processes that lead to the formation of substrates. The more recalcitrant lignin components are selectively preserved. In the second stage, the substrates and the preservation products are further transformed by synthetic processes, which result in the formation of humus. Oxidative coupling plays an important role in humus formation. The reaction can be catalyzed by either enzymes originating from microorganisms or abiotic catalysts such as clay minerals or metal oxides.

Humus is a substance that can be envisioned as a large polymer with aromatic and aliphatic moieties. Humic substances do not have a definitive structure; instead, they exhibit an open structure in a state of constant formation or modification. For this reason, any substance that chemically resembles a natural humus constituent can participate in humification and become incorporated into soil organic matter (Bollag *et al*. 1998). Organic compounds can be sequestered (Dec *et al*. 1997), adsorbed (Dec and Bollag 1997) or covalently bonded to humus (Park *et al*. 2000). Only if compounds or their metabolites are bound through covalent linkages are they considered to be an integral part of humus (Achtnich *et al*. 1999). Compounds that are bound chemically are considered irreversiblely bound because they cannot be recovered by methods that do not change the chemical character of the immobilized compounds (Dec *et al*. 1997). They are highly resistant to exhaustive extraction with organic solvents and release by microbial activity (Dec and Bollag 1988).

Several mechanisms are involved in the incorporation of organic compounds into humus. The

incorporation relies largely on the oxidative coupling reaction. In oxidative coupling reactions, aromatic compounds are oxidized to form free radicals or quinones. The unstable oxidation products are then subject to chemical coupling (Park *et al*. 1999). The chemical coupling not only happens among reactive free radicals, they also couple to molecules of relatively resistant compound (Roper *et al*. 1995). If the reaction takes place in polluted soils, they couple mainly to humus, which abounds in stable free radicals (Stevenson 1994), resulting in the formation of covalent linkage between humic substances and the xenobiotics or their degradation products (Bollag *et al*. 1992). Humus itself also exhibits catalytic properties due to the presence of free radical components (Stevenson 1994).

Sarkar *et al*. (1988) provided evidence that oxidative coupling is mediated by enzymes. In this study, ^{14}C-labeled 2,4-dichlorophenol, natural fulvic acid and four oxidoreductive enzymes were incubated. It was found that no binding was observed in control samples with boiled enzyme. In samples with non-boiled enzymes, however, substantial amounts of radioactivity were incorporated into fulvic acid. Due to the complexity of soil organic matter, model systems have been used for determining the types of chemical bonds and reaction mechanism involved. Typically a free enzyme is incubated with a single xenobiotic substrate in the presence of a humus constituent as co-substrate. Kim *et al*. (1998) studied reaction of herbicide bentazon and its metabolites with humic monomers in the presence of oxidoreductases. Transformation was monitored on the basis of disappearance of bentazon/metabolites from supernatant. It was found that the extent of transformation depended upon the chemical structure of the substrate, the type and concentration of co-substrate, and the pH of the reaction mixture. The difference in the reactivity of various substrates appears to be related to the coupling potential of the side groups. The hydroxyl group of hydroxy-bentazon is more reactive than a side chain such as a methyl group. Therefore, after incubation of 24 h, 100% of 6-hydroxy-bentazon was transformed in the presence of laccase and guaiacol at pH 3.0 while the transformation was 20% for des-isopropyl-bentazon under the same condition.

Park *et al*. (1999) revealed that the effect of humic constituents on the transformation of chlorinated phenols and anilines was determined by different transformation mechanisms. When

4-CP and catechol were incubated together with laccase, the former was oxidized with the formation of free radicals, while the latter formed phenoxide anions and o-quinones. Catechol was transformed at a higher rate than 4-CP. Because the oxidation products of 4-CP and catechol differed in their oxidized forms and the rate of formation, only 21.4% of 4-CP was transformed. On the contrary, when both 4-CP and syringaldehyde were oxidized to free radicals, 82% of 4-CP was transformed because 4-CP and syringaldehyde were on the same oxidized form and capable of efficient cross coupling.

To further approximate the conditions in soil, Park *et al*. (2000) studied chlorinated phenol transformation in the presence of humic acid. In this study, humic acid in many cases enhanced the incorporation of chlorinated pollutants into OM. The author explained the result by speculating that the chlorinated compounds were subject exclusively to oligomerization in the absence of humic acid. When humic acid was present, it underwent binding. However, the effect of humic acid also depended on the substrate and the type of catalyst. It was found that the transformation of 4-CA was only slightly affected by humic acid. This was due to the large oligomerization/low binding to humic acid.

Covalent binding can be achieved through other reactions. Dec *et al*. (1997a) investigated the soil bound residues of ^{13}C labeled fungicide cyprodinil by NMR spectroscopy. Soil bound residue was prepared by incubating 500 mg/kg of 2-pyrimidyl-^{13}C-and ^{14}C-labeled cyprodinil and soil. Control samples were amended with acetone and no cyprodinil was added. After 169 d incubation, soil samples were extracted to separate humic acid. Comparing the ^{13}C-NMR spectra for the humic acid extracted from soil after incubation with those extracted from soil without incubation, it was concluded that the cyprodinil molecule was cleaved between the aromatic rings. Phenyl and pyrimidyl moieties resulting from the cleavage were covalently bound to humic acid and became an integral part of humus. To identify whether the ^{13}C-labeled fragments were bound covalently or only entrapped in the molecular net of humic acid, Dec *et al*. (1997b) used a silylation procedure to characterize bound and sequestered residues of cyprodinil in soil. Silylation reaction involved the substitution of a silyl moiety for the hydrogen atom in various functional groups without further alteration of the derivatized molecule. Since replacement of the

active hydrogens in the soil matrix with silyl groups caused a disintegration of humic aggregates into smaller fragments, silylated humic materials were soluble in organic solvent and was suitable for high resolution in NMR. It was demonstrated that the phenyl and pyrimidyl moieties of the cleaved fungicide became integral parts of the humic acid matrix.

Thron *et al*. (1996) used ^{15}N-NMR spectroscopy to demonstrate covalent binding of ^{15}N-labeled aniline to humic acid when the two components were dissolved in water and stirred for 5 days at pH 6. The changes in the chemical shifts of the ^{15}N atom indicated that binding was due to nucleophilic addition reactions of aniline with the quinone or carbonyl groups typical for humic substances.

Ionic bond between organic compounds and humic substances is another mechanism involved in irreversible binding process. Senesi and Testini (1980) reported the formation of ionic bonds between protonated s-triazine and carboxylate anion.

2.3.2 Difference among Organisms

Whether or not sorbed contaminants can be directly available to microorganisms is still in debate. The most commonly accepted concept is that desorption is a prerequisite of biodegradation, i.e., the contaminants need to diffuse out of the sorbent first before they can be degraded by microorganisms. Recently, other researchers showed evidence that sorbed substrate may be directly available for degradation by attached cells (Feng *et al*. 2000; Park *et al*. 2001; Guerin and Boyd 1992; Calvillo and Alexander 1996; Tang *et al*. 1998; Grosser *et al*. 2000; Laor *et al*. 1996) All of the conclusions of degradation of sorbed compounds are based on the evidence that the biodegradation rate is higher than that calculated from instantaneous desorption (Feng *et al*. 2000) or part of sorbed compounds that can not be removed by abiotic desorption was bioavailable to bacteria (Park *et al*. 2000; Calvillo and Alexander 1996). Although no evidence of direct consumption of sorbed contaminant is provided, this evidence showed significant diversity among microorganisms in the abilities to degrade contaminants. These important biological traits in evaluating bioavailability include the ability to attach to the sorbent

surface and to survive on low substrate concentration (Scow and Johnson 1997).

Kinetic Parameters

Guerin and Boyd (1992) studied the ability of two bacterial species to degrade naphthalene. *Pseudomonas putida* 17484 (Pp17484) and NP-Alk were used to study biodegradation of naphthalene in the presence of soil. For NP-Alk, decreases in the rate and extent of naphthalene mineralization were observed with increases in the amount of added soil. Sorbed naphthalene was not utilized by the cells. In contrast, Pp17484 showed a rapid exponential phase followed by a linear phase of $^{14}CO_2$ production. A portion of sorbed naphthalene was also available to the organisms. In a later paper (Guerin and Boyd 1995), the authors found Pp17484 maintained naphthalene degradation ability at low levels for long periods of time in the absence of naphthalene and could rapidly respond to new additions of naphthalene to culture. In contrast, the NP-Alk was unable to maintain naphthalene degradation activity in the long-term absence of naphthalene and needed many hours after exposure to naphthalene before degradation activity was induced. Gueer and Shelton (1992) examined two *Alcaligene* sp strains with similar maximum growth rates but with 10 fold difference in the half-saturation constant K_s. When concentration of 2, 4-D was high (60 and 600 μg/ml), the strain with the lower K_s, could use 2, 4-D more rapidly than the strain with the higher K_s. It is speculated that the half-saturation constant influences the strain's degradation ability in a substrate-limited environment.

Feng *et al*. (2000) investigated the bioavailability of soil-sorbed biphenyl to *Pseudomonas putida* P106 and *Rhodococcus Erythropolis* NY05. Although strain NY05 had a much lower K_s value than P106, the possession of low K_s did not give NY05 the competitive advantage for sorbed biphenyl. To explain the difference between two strains, the author proposed the substrate acquisition mechanism. The hydrophobic molecule sorbed on the surface may dissolve in the lipids at the bacteria surface before entering the cytoplasm. The gram-positive bacterium NY05 has 90% of peptidoglycan. The gram-negative bacterium P106 has an additional lipopolysaccharide layer besides the peptidoglycan, which contributed to the dissolution of HOCs to lipids at the bacteria surface.

Attachment

The attachment of bacteria to sorbents likely influences their biodegradation ability. Although there appears to be a qualitative consensus that surfaces influence bacterial metabolism, the experimental observations are not always consistent; neither has a general explanation been advanced for this influence. Even for the same experimental result, researchers are arguing the different mechanisms behind the phenomena. A positive influence of surfaces on bacterial activity was found when marine bacteria were used to degrade amino acid (Bright and Fletcher 1983). The author attributed it to the accumulation of substrate at the surface of sediments.

Calvillo and Alexander (1996) compared the biodegradation of cells with different ability to attach to sorbents. Cells, which can attach to the surfaces of sorbent, showed high mineralization extent after 150 hrs. It was speculated this difference of utilization might involve lipids at the bacterial surface, the hydrophobic molecules on the solids dissolved first into these lipids before entering the liquid phase. It was also found that 80% of 120 μg of biphenyl sorbed to the beads were mineralized by consortium SBP-1. But none of the twelve bacteria isolated from the consortium could mineralize sorbed biphenyl. The mechanisms involved in this phenomenon were not fully determined in this investigation. Harms and Zehnder (1994) used porous Teflon beads to study the degradation of sorbed 3-chlorodibenzofuran by a *Sphingomonas* sp. and found that attached cells degraded the bound chemical more rapidly than could be accounted for by the rates of desorption into the aqueous phase.

Enrichment Techniques

Tang *et al*. (1998) used different enrichment techniques to isolate phenanthrene-degrading bacteria. Bacterium P5-2 and consortium NL-W were grown on phenanthrene sorbed to biobeads. It was shown sorbed phenanthrene was degraded faster and to a greater extent by P5-2 and NL-W than bacteria P3 and RB-P, which were isolated on nonsorbed substrate. Possible mechanisms involved were production of surfactants or direct utilization of sorbed chemicals by attaching to the solids. Grosser *et al*. (2000) selected bacteria in the presence of a sorptive phase. When selected strain SM7.6.1 was incubated in the presence of sorptive phase, it was found that the mineralization extent of SM7.6.1 was higher than other bacteria that were not enriched under

the same condition (C4.7, S2.1). The author explained this difference by suggesting the strain SM7.6.1 was better adapted to sorptive environments than other isolates.

2.4 Aerobic Toluene Degradation

The oxidation of toluene can be initiated by insertion of O into any of the four unique C-H bonds in this molecule (Fig. 2.1). Toluene 4-monooxygenase (T4MO) catalyzes reaction from toluene to *p*-cresol (4-hydroxytoluene). Similarly, Toluene 3-monooxygenase (T3MO) produces *m*-cresol (3-hydroxytoluene) and Toluene 2-monooxygenase (T2MO) produces *o*-cresol (2-hydroxytoluene). Xylene monooxygenase (XMO, also toluene side chain monooxygenase) hydroxylates the methyl carbon to form benzyl alcohol. In addition, toluene dioxygenase (TDO) can insert both atoms of oxygen into toluene to form *cis*-dihydrodiol (Arp *et al*. 2001).

Bacteria have diverse metabolic capabilities to degrade toluene. Different toluene oxidizers and their kinetic parameters are summarized in Table 2.4. *Pseudomonas putida* F1 (*PpF1* ATCC: 700007) was selected in this study because of it has been subjected to extensive study (Gibson *et al*. 1968a; 1968b; 1970; 1989) and it has fast toluene biodegradation rate. The bacterium *pseudomonas putida* 39D was isolated from soil by selective culture with ethylbenzene as the carbon source (Gibson *et al*. 1968a). Its ability to degrade toluene, benzene, isopropylbenzene was also detected. Its degradation pathway (TDO pathway) was first demonstrated by Gibson *et al*. (1970), as shown in Fig. 2.1. This strain was later designated *P. putida* F1 (Gibson *et al*. 1989). According to Stanier *et al*. (1966), *PpF1* is unicellular rod, gram negative. They are motile by means of one or more polar flagella and do not form spores, stalks or sheaths. The enzyme involved in toluene degradation is TDO, a multicomponent dioxygenase composed of three separable protein components (Zylstra and Gibson 1989). TDO is a versatile enzyme with a relaxed specificity, which allows the organism to use a wide range of aromatic compounds such as benzene, ethylbenzene, phenol, and benzyl alcohol as carbon source (Gibson and Parales 2000; Gibson *et al*. 1968a).

TABLE 2.4 Model Parameter for Aerobic Toluene Biodegradation by Pure Cultures

Bacteria	T (°C)	max. conc.[b] (mg/L)	μ_{max} (h^{-1})	K_s (mg/L)	K_i[d] (mg/L)	$Y_{X/S}$[e] (g/g)	Reference
P. p F1	30	43	0.86± 0.01	13.8±0.9	/	1.28±0.13	Reardon *et al*. 2000
P. p R1[a]	25	4	0.504	0.1	/	1.2	Pedersen *et al*. 1997
P. p. 54G	24	50	0.42±0.05	3.98±0.78	42.78±3.87	0.90±0.13	Mirpuri *et al*. 1997
P. p. O1	30	70	0.72	15.07	44.33	0.64	Oh *et al*. 1994
P. fragi B1	room	10	0.543±0.076	1.96±0.91	/	1.22±0.1	Chang *et al*. 1993
P. sp. X1	room	10	0.452±0.115	1.88±1.26	/	0.99±0.25	Chang *et al*. 1993
P.p. 23973	32	30	0.437	6.0	1980	/	Chio *et al*. 1992
P. p mt-2	/[c]	/	/	0.708±0.156	/	/	Duetz *et al*. 1998
B. cepacia G4	/	/	/	2.35	/	/	Arp *et al*. 2001

[a] A value of 0.048 h^{-1} for the specific decay rate was also reported. [b] Maximum toluene concentration. [c] Not available. [d] Parameters are for the Monod model unless a value of K_i is given, in which case the Andrews model was used. [e] The theoretical value of $Y_{X/S}$ for growth on toluene is 1.23 g/g.

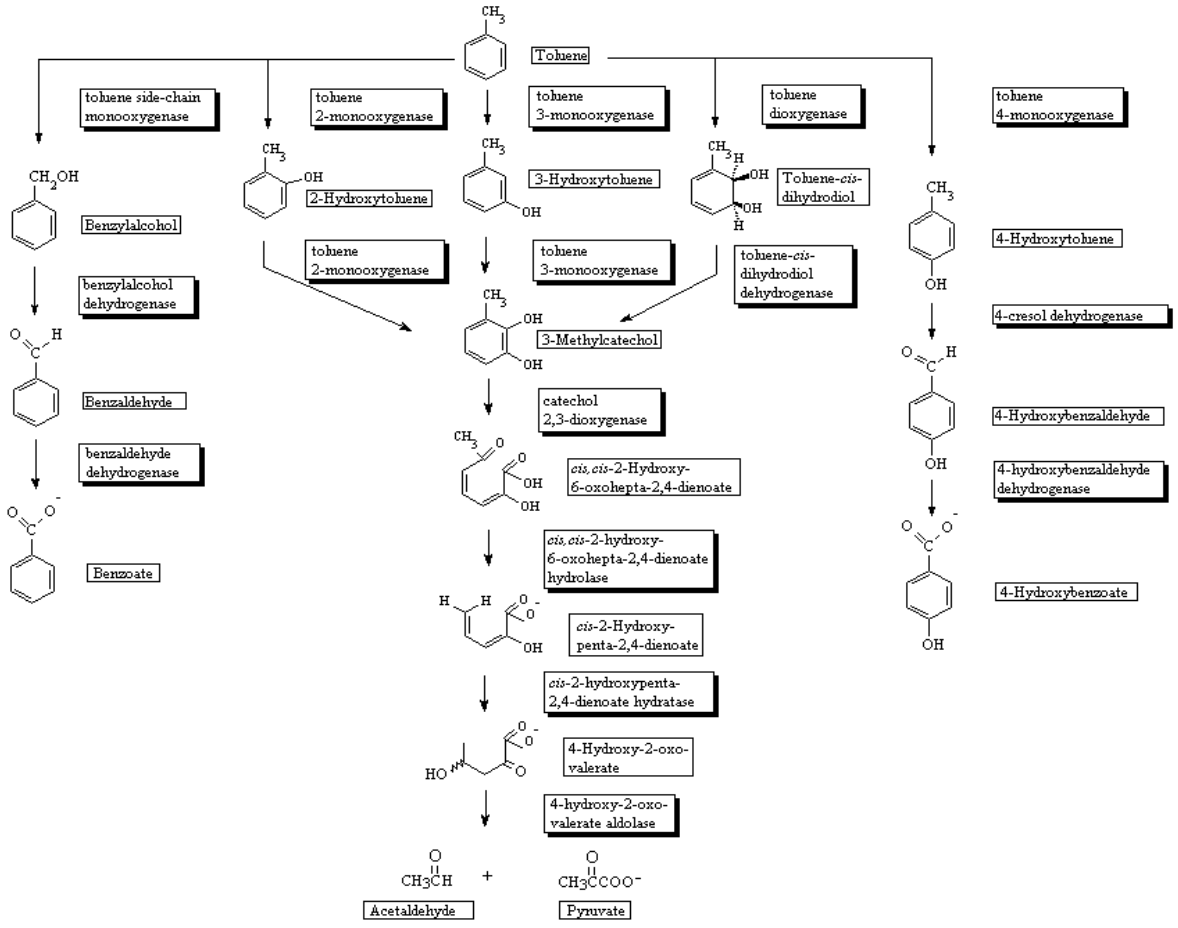

FIGURE 2.1 Toluene pathway map.

source: http://umbbd.ahc.umn.edu/tol/tol_map.html

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Chapter 3

Method Development

In this chapter, experimental techniques used to evaluate bioavailability of toluene sorbed to MSW are described in detail. Preliminary data are included to give an overview of the development and gradual improvement of the experimental techniques.

3.1 Anaerobic Bioavailability Test

The objective of this test is to evaluate the potential of using consortia enriched from landfills in assessing bioavailability of HOCs sorbed to MSW component. The description of materials also applies to the other experiments in this chapter.

3.1.1 Materials

Sorbents and Their Characterization

Due to the complexity and heterogeneity of MSW, bioavailability tests are conducted with the major organic MSW components in pure form. Rabbit food (Manna Pro® Corporation, St. Louis, MO) contains both simple and complex carbohydrates as well as proteins in the form of alfalfa, wheat, soy and oat products, and it was used to represent food and yard waste. Poly (vinyl chloride) (PVC) (Sigma-Aldrich Milwaukee, WI) and high-density polyethylene (HDPE) were tested to represent glassy and rubbery polymers respectively. Newsprint and office paper were chosen to represent the major paper types in MSW.

Newsprint was collected from The News & Observer Recycling Division (News & Observer Publishing Co., Garner, NC). Office paper was collected from the NC State University recycling center. Office paper, newsprint and rabbit food were prepared as fresh and anaerobically degraded form to test the effect of refuse decomposition. All the sorbents except PVC were then dried and ground to powder by Thomas-Wiley laboratory mill and stored in desiccator until use.

The characterization of sorbents is summarized in Table 3.1 (Wu *et al*. 2001).

Sorbate

^{14}C Labeled toluene (2.8 mCi/mmol, 99% purity) was purchased from Sigma Chemical Co. (St. Louis, MO). For stock solutions, 1 mCi radioactive toluene was mixed with 10 ml non-labeled toluene (Reagent grade, Fisher Scientific, Pittsburgh, PA) to give a final concentration of 150325.33 dpm/μL. Stock solutions are kept in a freezer at –10 ºC until use.

Leachates

Acidogenic leachate was produced by recirculating water through fresh residential refuse. The pH of the leachate was 4.9 and COD was around 22,000 mg/l. The acidogenic leachate has high concentrations of volatile fatty acids (VFA), which are summarized in Table 3.2. Methanogenic leachate was generated in the laboratory from decomposed refuse with pH around 8 and TOC around 120 mg/l.

3.1.2 Methods

Prior to each test, test materials were sterilized by ^{60}Co gamma irradiation. Methanogenic leachate was filter-sterilized through 0.22 μm nylon filter. To begin a test, the appropriate mass of a material was added to the serum bottle in triplicate. The materials were weighed out in an aluminum pan that had been wiped with ethanol and flamed to sterilize it. After the materials had been added to serum bottles, sterile, anaerobic methanogenic leachate was added to the bottles in anaerobic hood until bottles were filled to minimal headspace. Bottles were spiked with the appropriate volume of ^{14}C compound to obtain an activity of 2 μCi ^{14}C. The bottles were then sealed with the Teflon™ coated butyl rubber stoppers (West Company, Lionville, PA) and aluminum crimp caps. Six control bottles were included with leachate and no solids to quantify nonsorptive losses. After addition of ^{14}C test compound, bottles were aged in a tumbler for the duration of the aging period.

TABLE 3.1 Characterization of Sorbents [a]

Sorbent	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Lipophilic extractives (%)	Crude protein (%)	Ash (wt%)	f_{oc}	BET surface area (m^2/g)
Poly (vinyl chloride)	N/A	N/A	N/A	N/A	N/A	0.00	0.389	0.8
High density polyethylene	N/A	N/A	N/A	N/A	N/A	0.01	0.876	0.6
Fresh office paper	64.7	13.0	0.93	0.7	0.31	11.6	0.373	2.8
Degraded office paper	36.2	6.9	4.8	3.3	4.99	38.4	0.278	6.0
Fresh newsprint	48.3	18.1	22.1	1.6	0.44	2.0	0.451	2.6
Degraded newsprint	35.1	16.0	32.3	1.4	3.74	6.4	0.455	3.4
Fresh rabbit food	30.6	15.4	9.5	4.9	18.1	7.7	0.423	0.6
Degraded rabbit food	7.1	5.7	25.2	4.5	20.6	34.5	0.329	0.5

[a] Values are averages of replicate analyses. [b] N/A: not analyzed

TABLE 3.2 VFA Analysis of Acidogenic Leachate [a, b]

Sample number	acetic acid	propionic acid	i-butyric acid	Butyric acid	2-methyl butyric acid	i-valeric acid	Valeric Acid	i-caproic acid	caproic acid	heptanoic acid
1	1658	1571	101	3862	52	61	1774	22	337	ND
2	1886	1675	141	3736	56	66	1717	21	317	ND

[a] Sample 1 and 2 were duplicate acidogenic leachate samples. [b] Concentration in mg/L.

Following aging, bottles were removed from the tumbler. Duplicate 0.5 ml samples were taken from the aqueous phase. This step quantified the amount of test compound sorbed to the solid. After sample removal, as much additional liquid as possible was removed from bottle using a disposable syringe. The volume removed was replaced with inoculum from a culture known to degrade o-xylene (enriched from contaminated sediment obtained from a sandy zone of an aquifer in Pensacola, FI. (Edwards and Grbic-Galic 1994)), leaving a 10 ml headspace for gas accumulation. The concentration of the test compound was adjusted using the ^{14}C stock solution to be the same in the inoculum as in the aqueous phase of the test bottles to prevent any additional sorption and desorption.

For the six control bottles, three were inoculated with the same amount of microorganisms. The inoculum was autoclaved and added to the other three abiotic control bottles. These three abiotic control bottles were set up to monitor the abiotic loss of the test compound during incubation.

Following inoculation. Three positive controls, three bioavailability tests and three abiotic controls were maintained at 37°C for 4 months. Gas production was monitored by venting the headspace of bottles directly into gasbags. $^{14}CH_4$ and $^{14}CO_2$ and volatile ^{14}C-test compound were quantified using gas trapping protocol.

3.1.3 Experimental Results

The bioavailability result was shown in Table 3.3 (Pelton 2000). The cumulative o-xylene recovery was low in all bottles over 1 month monitoring period. The overall recovery for bioavailability bottles were around 1% with majority of the recovery representing volatilization of o-xylene, which was trapped on the ORBO™ tubes. The biodegradation rate in biological control bottles was also very low. Of the 7% recovery, less than 10% is $^{14}CH_4$ and $^{14}CO_2$. The recovery of volatile o-xylene on the ORBO™ tubes during each trapping period verified that there was o-xylene available to the bacteria in each bottle, but it was hard to argue that there was degradation occurring based on extremely low $^{14}CH_4$ and $^{14}CO_2$ produced.

TABLE 3.3 Anaerobic Bioavailability of o-xylene Aged One Month on Degraded Newsprint

Bottle number		Initial dpm added	dpm removed with leachate	dpm added with inoculum	dpm trapped				Cumulative $^{14}CO_2$ $^{14}CH_4$ recovery (%)	Cumulative recovery (%)
					$^{14}CO_2$	$^{14}CH_4$	ORBO	Total		
Bioavail-ability	1	1062959	94197	50334	0	0	19223	19223	0	1.89
	2	1036385	88498	50334	0	986	14140	15126	0.099	1.52
	3	1062959	81867	50334	571	0	17017	17588	0.055	1.76
Positive control	1	1062959	0	0	301	2115	71320	73736	0.23	6.94
	2	1062959	0	0	536	6483	73386	80405	0.66	7.56
	3	1062959	0	0	324	2211	80858	83273	0.23	7.85
Abiotic Control	1	1062959	90146	54317	0	0	9542	9542	0	0.93
	2	1062959	92985	54317	0	0	9645	9645	0	0.94
	3	1062959	98349	54317	0	0	10378	10378	0	1.02

3.1.4 Conclusions and Recommendations

1 O-xylene is a volatile compound. Previous test showed that significant loss occurred when using gray Teflon™ coated butyl rubber stopper to seal serum bottle during long time aging. The loss during aging will introduce uncertainties into the mass balance. To avoid this, the bottles should then be sealed in a way that allows for the headspace to be vented without opening the bottle.

2 After three months of incubation. It was found that large amount of gas was produced in abiotic control bottle; indicating γ irradiation prior to loading the bottle was ineffective. Contamination may occur during weighing and spiking procedures.

3.1.5 Improvements

Based on the anaerobic bioavailability test, the experimental design was improved in the following aspects.

1 Serum bottles with Teflon™ coated rubber stoppers were replaced by 160 ml flame-sealable ampoules (Prism Glass, RTP, NC) to avoid volatile loss. After placing the solid and liquid into the bottle and spiking toluene, the bottle was quickly flame sealed with a hand torch.

2 Although it is ideal to study bioavailability in landfill under anaerobic conditions, an aerobic

system was used considering low $^{14}CO_2$ and $^{14}CH_4$ production in anaerobic tests. It is well accepted that sorbed compounds cannot be degraded without being released to the aqueous phase. Therefore bioavailability of organic compounds is limited by desorption instead of types of microorganisms.

3 Much higher toluene concentrations are used although the typical toluene concentration in landfill leachates is between 0-7500 μg/l (Christenssen *et al*. 1994). The reasons are: the flux of energy from degradation of insufficient substrate cannot sustain the biomass; in lower substrate condition, the concentration may not be sufficient to induce enzyme production. In addition, following the bioavailability test, a series of extractions will be performed to determine the association of non-bioavailable compounds with solids. Lower initial concentrations make HOCs hard to detect during these analyses.

3.2 Aerobic Bioavailability Test

The objective of this test is to evaluate the potential of using redesigned aerobic system in assessing bioavailability of toluene in MSW landfill.

3.2.1 Methods

Sample Aging

Solid materials were weighted into 160 ml ampoule; 60 ml of growth medium was then added to wet the solid. 20 μl of stock solution, which contained approximately 3×10^6 dpm of labeled toluene was spiked to the bottom of each ampoule, resulting in a final liquid phase toluene concentration of 100-200 mg/l. The ampoule was quickly flame sealed to avoid volatilization loss. The flame-sealed samples were sterilized with 2.2 Mrad of gamma irradiation from a ^{60}Co source. Then samples were completely mixed in a New Brunswick Innova 2300 shaker (Edison, NJ) for different time periods.

Bacterial Strains and Growth Conditions

Strain *Pseudomonas putida* F1 (from Dr. Mike Hyman, North Carolina State University,

Raleigh, NC) was grown at 30±1°C in medium containing H_3BO_3 1.43 mg, $ZnSO_4 \cdot H_2O$ 0.32 mg, $CoCl_2 \cdot 4H_2O$ 0.1 mg, $CuSO_4 \cdot H_2O$ 0.08 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.05 mg, KH_2PO_4 4.2215 g, K_2HPO_4 3.368 g, $CaCl_2 \cdot 2H_2O$ 0.05 g, $FeCl_3$ 0.005 g, $MgSO_4 \cdot 7H_2O$ 0.201 g, NH_4NO_3 0.5 g, disodium EDTA 0.01 g per liter of D. I. water. To prepare an inoculum of this bacterium, 25 ml sterile medium was inoculated and the organisms were grown in an incubator shaker (30±1°C, 150 rpm) with 12.5 µl toluene supplemented as substrate. After 24 hr, 0.5 ml of the culture was transferred into another 25 ml of sterile growth medium and incubated for 24 hr. The cells were washed twice and resuspended in NaH_2PO_4-Na_2HPO_4 pH 7 buffer before use.

Bioavailability Test

The bioavailability of a pollutant to bacteria can be assessed by monitoring the degradation of toluene. In this study, a pure culture of *PpF1* was used as toluene degrader. While this pure culture system lacks the complexity of natural landfill communities or those in soil, it is a reasonable starting point for the development and validation of desorption and bioavailability processes.

The apparatus for bioavailability testing is shown in Fig 3.1. After the compounds had aged for 1 day, 1 month, 6 months and 1 year, triplicate samples were broken at the score mark. One ml of aqueous phase was quickly withdrawn to measure toluene concentration. Duplicate solid-free blank sample was then prepared by adding stock solution to attain the same liquid phase concentration as aged samples. The triplicate samples and two blanks were inoculated with 3 ml of a suspension of a toluene mineralizing bacterium; strain *PpF1* to give 1.5×10^8 cells/ml. Cell number was determined by optical density of the culture according to the CFU-optical density calibration curve (Fig C-1). After inoculation, the bottle was sealed with a stainless Swagelok™ cap. Three stainless steel needles, one 18G, 2 inches, two 18G, 6 inches were welded through the top of the cap beforehand. Two sterile 12×75 mm glass vials were attached to the two long needles and put into liquid phase. The ends of the two needles were kept in touch with the bottom of glass vials. 2 ml of 2 N NaOH was injected into one vial as absorbent of evolved $^{14}CO_2$, 1 ml of 30% hydrogen peroxide and 0.5 ml of $FeCl_2$ were injected to the other vial to

provide oxygen for bacteria growth. It was verified that $FeCl_2$ can catalyze the decomposition of hydrogen peroxide and the rate of decomposition is concentration dependent (Schumb 1955). Since BOD of solid materials are quite different, the concentration of the catalyst was also changed in order to meet the oxygen demand of both sorbent and substrate. Two push button syringe valves were attached to the hubs of the stainless steel needle to prevent volatilie loss.

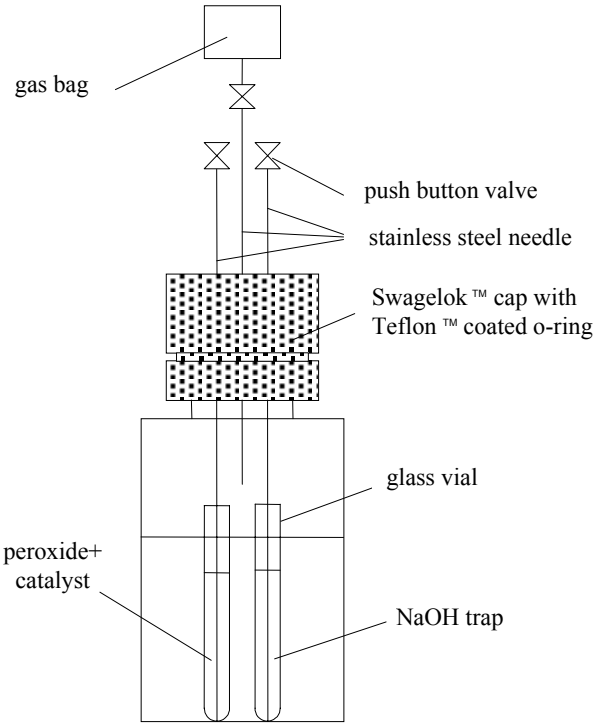

FIGURE 3.1 Experimental design for aerobic toluene bioavailability test.

Glass vial dimension 10×75 mm, two long stainless steel needles: 18G, 15cm. One short stainless steel needle: 18G, 5cm.

Supplying oxygen through a double layer Tedlar gasbag full of oxygen instead of using peroxide was also tested. Once the oxygen in the ampoule was depleted, the vacuum formed inside would suck oxygen into the ampoule from the gasbag. The experimental result was unsuccessful. The vacuum formed could not overcome the resistance along tubings as well as connectors between the ampoule and the gasbag. Not enough oxygen was detected in ampoule (< 1 mg/L). Therefore, peroxide was still used as oxygen source, the Tedlar gasbag was kept to collect any excess oxygen produced by hydrogen peroxide. The bioavailability bottles were incubated at 30±1°C in

an incubator shaker (New Brunswick Scientific C24 Classic series, Edison, NJ) operating at 130 rpm. Periodically, the NaOH was withdrawn and fresh NaOH was injected through the needle and $^{14}CO_2$ was determined by adding 6 ml of scintillation cocktail and counted by scintillation counter (TRI-CARB 2100TR Packard Instrument Company, Downers Grove, IL). Because of high base content of the sample, they were always stored overnight in refrigerator before analyzing to reduce the effect of chemiluminescence. Hydrogen peroxide was replaced in the same way. To maintain a DO level higher than 3 mg/L, gas phase O_2 concentration is monitored by GC. The frequency of H_2O_2 replacement was determined by the oxygen demand of different materials.

Analysis of Residual ^{14}C at Termination of Bioavailability Test

After the bioavailability test, the bioavailability samples are subjected to a series of extractions to analyze the residual ^{14}C distribution (Fig 3.2). Triplicate samples of each material were centrifuged at 3000 rpm for 10 min (Eppendorf centrifuge 5810, Brinkmann Instruments Inc., Westbury N.Y.). Supernatant was removed by decanting from solids and analyzed for dissolved ^{14}C. Initially, 25 ml benzyl alcohol was added to the solid. Sorbent and benzyl alcohol were mixed and shaken for three days. Benzyl alcohol extractions were repeated until no more counts were recoverable from the solid. The samples were washed with D. I. water several times to remove benzyl alcohol and then subjected to a series of NaOH extraction until the concentration of the extract was lower than 100 dpm/ml. 0.1 N NaOH extracts were combined.

NaOH extracts were acidified to pH 1.5 by 6 N HCl to precipitate humic acid. The precipitate was collected by centrifugation and resuspended in NaOH. Duplicate 0.5 ml samples were taken to measure radioactivity and TOC (Shimadzu TOC 5000A analyzer, Columbia, MD), humic acid fraction was then fractionated by HPSEC according to different molecular weight.

The supernatant from the pH=1.5 acidification step was passed through a 54 ml glass column packed with 50 ml DAX8™ resin ($k'_{0.5r}$ = 5). Flow rate was 12.5 ml/min. Fulvic acid, which was retained on the resin, was eluted with 400 ml of 0.1 N NaOH at 1.5 ml/min by peristaltic pump. Effluent was collected and fractionated by HPSEC. Duplicate 0.5 ml sample was taken to

determine radioactivity. Nonsorbed effluent was non-humic substance; samples were taken to measure TOC and radioactivity.

Undissolved humin was collected and that part was termed as humin and burned in tube furnaces at 875°C; $^{14}CO_2$ was trapped in four successive sodium hydroxide traps.

3.2.2 Experimental Results

The results of aerobic bioavailability are shown in Fig 3.4 and Fig 3.5. The extent of biodegradation varied markedly among sorbents and was correlated with the sorbent's chemical characteristics (Table 3.4). In general, toluene bioavailability was reduced by aging. Analysis of the solid phase at the termination of bioavailability tests revealed that ^{14}C was present in all three major forms of humic substances; humic acid, fulvic acid and humin. High-performance size exclusion chromatography showed that ^{14}C in humic and fulvic acid fractions was present in molecular size fractions greater than that of free toluene. However, it was also observed that after γ-irradiation approximately 46% of the added toluene was converted from a spargeable compound to a mixture of non-spargeable materials (Table 3.4). The mechanisms of reactions occurred during irradiation of toluene were reported by Weiss and Rao (1965), Weiss and Collins (1964, 1966). As a result, it is apparent that ^{14}C-toluene was not the only initial compound leading to the results with γ-irradiation presented in Table 3.4.

TABLE 3.4 Comparison of Toluene Bioavailability with and without Gamma Irradiation [a]

Sorbent	Aging time (d)	Gamma irradiation	Sorbed (%)[b]	$^{14}CO_2$ (%)	Particulate and cell mass (%)	Non-volatile ^{14}C in liquid (%)	Solvent extractable (%) [c]	Total Recovery (%)
HDPE	1	Yes	46.50 (2.01)	63.05 (1.11)	14.43 (0.79)	14.70 (0.49)	6.29 (0.07)	98.00 (1.01)
HDPE	1	No	76.51 (0.72)	71.12 (1.89)	17.07 (0.67)	2.34 (0.11)	[d]	[d]

[a] Data are averages of triplicate samples. Standard deviations are given in parentheses. Percentages are based on total amount of toluene added initially corrected for volatile loss. [b] Radioactivity in solid phase before inoculation. Percentages are based on total amount of toluene without correction of volatile loss. [c] Solvent is benzyl alcohol. [d] Not analyzed.

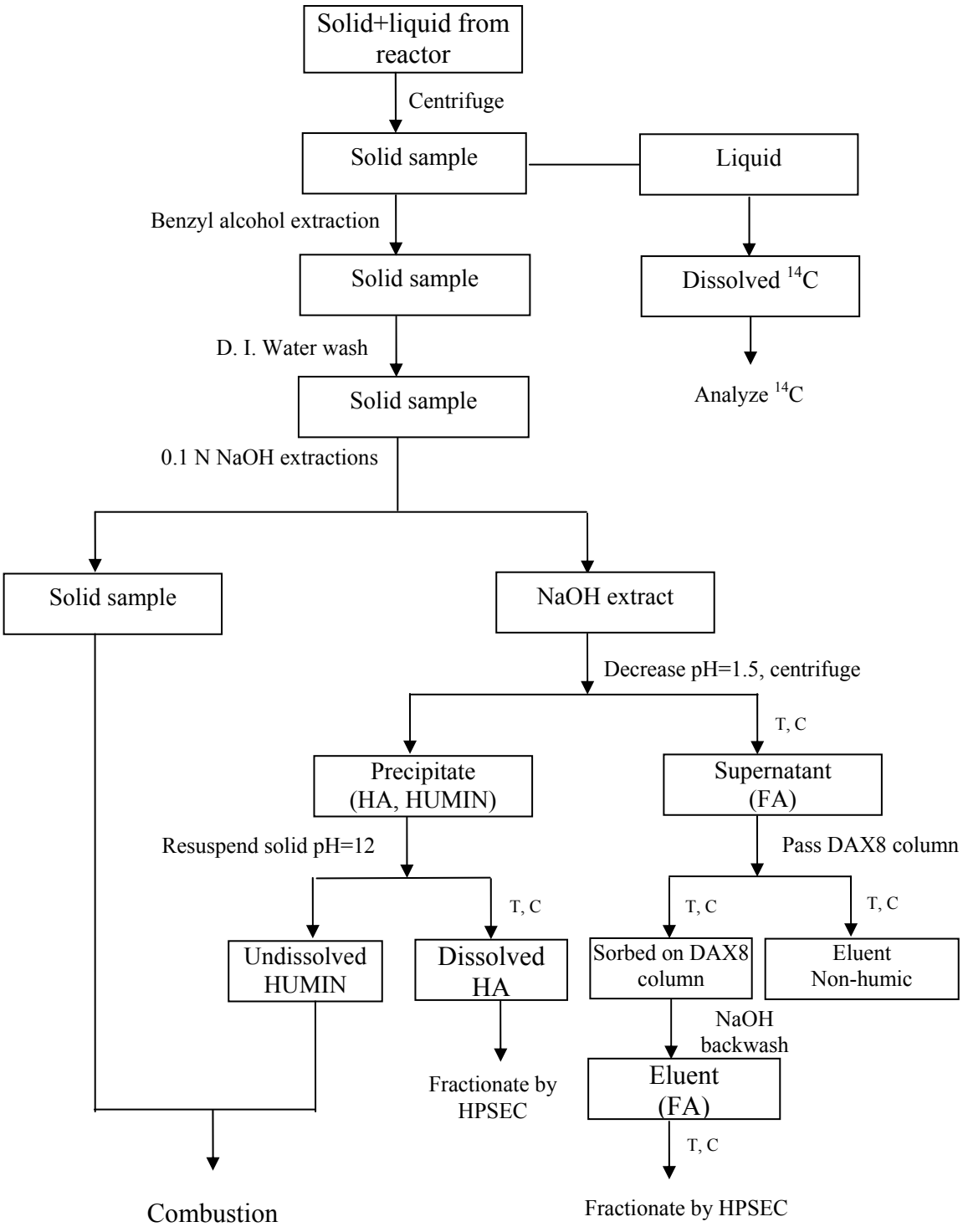

FIGURE 3.2 Analysis of residual ^{14}C after termination of bioavailability test.

T: Measure total organic carbon. C: measure radioactivity.

3.2.3 Improvements

Aging protocol was revised to eliminate the γ-irradiation of toluene. Sorbents were sterilized 2.2 Mrad of gamma irradiation from a ^{60}Co source. To each sorbent, 60 mL of sterile growth medium was added aseptically in the laminar flow hood. Upon the addition of toluene stock solution with sterile micro-liter syringe, the ampoules were quickly flame sealed and aged for 1, 30, and 180 days. No modification was made for other experimental techniques.

3.3 Preliminary Test

3.3.1 Toxicity Assay of Acidogenic Leachate

Acidogenic leachate has high concentrations of carboxylic acids as shown in table 3.2. To conduct bioavailability tests in acidogenic leachate, it is necessary to first evaluate its toxicity to strain *PpF1*.

Methods

500 ml acidogenic leachate was adjusted to pH 7 by 50/50 (w/w) NaOH. After precipitation, the leachate was filtered through 0.45 μm filter to remove particulates. Chemicals (trace minerals, K_2HPO_4, KH_2PO_4 were added according to medium formula to make the acidogenic leachate suitable for *PpF1* growth. The leachate was then sterilized by 2.2 Mrad of gamma irradiation from a ^{60}Co source. Triplicate undiluted 50 ml sterile acidogenic leachate samples were added to 250 ml sterile Erlenmeyer flasks (Fig 3.3). Triplicate acidogenic leachate samples were diluted to 50% of original concentration by sterile D. I. water. Another set of triplicate samples was diluted to make the final concentration 75% of the original leachate. The nine flasks were then inoculated with *PpF1* and incubated at 30±1 °C. Peroxide was changed daily as oxygen source. Oxygen concentration in aqueous phase was checked with Chemets self-filling dissolved oxygen ampoule (CHEMetrics Inc., Calverton, VA) to make sure oxygen concentrations were greater than 3 mg/L. This concentration was selected based on the research of Shuler and Kargi (1992) and Chiang *et al*. (1989), who found a threshold DO concentration of 2 mg/L and 0.4 mg/L respectively exist for aerobic BTX degradation. CO_2 produced was trapped in 10 ml 1 N CO_2

free NaOH. Growth of the bacteria was monitored by measuring the inorganic carbon in NaOH trap with TOC analyzer (Shimadzu TOC 5000A). Two blanks without inoculation were also prepared to monitor abiotic background.

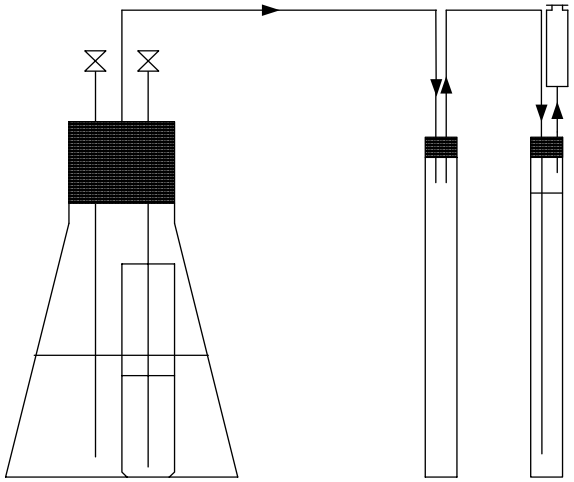

FIGURE 3.3 Experimental design for acidogenic leachate toxicity assay

Experimental Results

Most leachate toxicity assays that concluded acidogenic leachate was toxic were based on short-term experiments, which lasted 1 or 2 days. However, this study showed acidogenic leachate was not toxic to *PpF1* (Fig 3.4). Lag period was present in biodegradation, this may be because the inoculum was grown with toluene as substrate, and it took time for bacteria to develop enzymes to degrade carboxylic acids. Lag period was found proportional to the concentration of leachate. After 48 hours, bacteria acclimated to high concentration of carboxylic acid and began to metabolized leachate quickly.

Due to its high COD (around 22,000 mg/l), it will be very difficult to meet the necessary oxygen demand in acidogenic leachate in a sealed system. Because of this, bioavailability test will not be performed in acidogenic leachate.

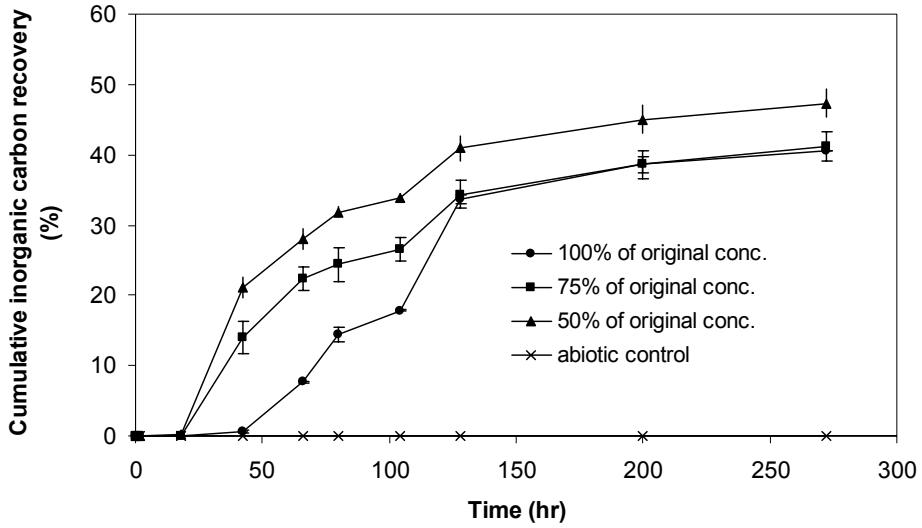

FIGURE 3.4 Toxicity assay of acidogenic leachate.

Data are averages of triplicate samples. Error bars represent standard deviations.

3.3.2 Bioavailability Test in Methanogenic Leachate

The objective of this test is to evaluate the potential of conducting bioavailability tests in methanogenic leachate

Materials and Methods

60 ml methanogenic leachate was adjusted to pH 7 by adding 6 N HCl. The leachate was then filtered through 0.45 μm filter to remove particulates. Chemicals (trace minerals, K_2HPO_4, KH_2PO_4 were added according to medium formula to supply necessary nutrients for *PpF1* growth. 60 ml filtered methanogenic leachate was added to 100 ml ampoule together with 3 g HDPE. 10 μL toluene stock solution was spiked. The ampoules were quickly flame sealed and sterilized by 2.2 Mrad of gamma irradiation from a ^{60}Co source. Duplicate blank samples with only methanogenic leachate and toluene in ampoule were prepared the same way. After aging, the four ampoules were then inoculated with *PpF1* and incubated at 30±1 °C. Peroxide was changed daily as oxygen source. $^{14}CO_2$ produced was trapped in 3 ml 2 N NaOH and analyzed

with liquid scintillation counting (LSC).

Experimental Results

Fig 3.5 showed the biodegradation rate of toluene in methanogenic leachate. Data are averages of duplicate samples. The y-axis is the percent of $^{14}CO_2$ recovery based on toluene in aqueous phase at the end of the aging period. Biodegradation of toluene in methanogenic leachate and leachate with HDPE was fast at the initial stage. In solid free leachate, the degradation leveled off after the leachate was exposed to oxygen for twenty hours. The $^{14}CO_2$ recovery was much lower than that in growth medium (70%). Samples of the methanogenic leachate at the end of biodegradation were counted by LSC. It was found there were still 2,000 dpm/ml ^{14}C compounds. The radioactivity did not change after sparging with N_2 for two hours, indicating there was no ^{14}C toluene present.

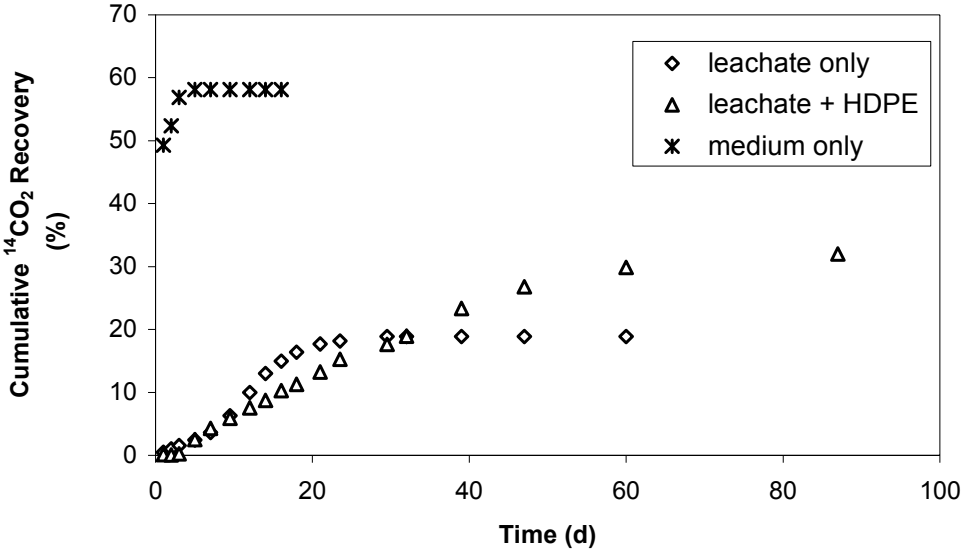

FIGURE 3.5 Bioavailability test conducted in methanogenic leachate.

To analyze the non-bioavailable radioactive compounds in methanogenic leachate, leachate after biodegradation was fractionated by HPSEC (Fig 3.6). Because of the complexity of the aqueous phase, which contained both high-molecular weight substances from methanogenic leachate and intermediates from toluene metabolism, it is not possible to identify the exact substances based

on the HPSEC analysis. But free toluene was no longer present since no peak showed up at 16 min, the elution time for aqueous toluene.

Several mechanisms can explain this test result. Toluene itself may form strong bonds with higher molecular weight organic matter in methanogenic leachate when oxygen was present. Once toluene was bound, it was no longer bioavailable to the bacteria. The other possibility was some hydroxylated intermediates were produced. The extracellular accumulation of metabolites in aqueous phase may be oxidatively coupled to organic matter in methanogenic leachate and could not be further degraded. Burgos *et al*. (1996) found similar phenomena when he investigated irreversible binding of naphthalene and α-naphthol to soil. Both oxic-autoclaved and anoxic-biotic system did not show irreversible binding. Only in oxic-biotic condition when oxygen and the appropriate extracellular enzymes were present the addition of a hydroxyl group to the aromatic ring made those compounds more reactive with soil organic matter.

Degradation in methanogenic leachate with HDPE kept increasing at lower rate. The presence of solid provided advantage for bacterial growth. In aquatic environments, it is well accepted that bacteria are most often associated with the solid surface. When attached to the solid surfaces, the bacteria were able to consume toluene that diffused out of HDPE before it was exposed and bound to leachate. With sorbed toluene as continuous substrate supply, the degradation continued. Assuming bacteria readily consumed toluene, the concentration gradient between solid phase (HDPE) and bacteria surfaces decreased as toluene continued to diffuse out of HDPE. This explained the gradually decline in degradation rate as time elapsed.

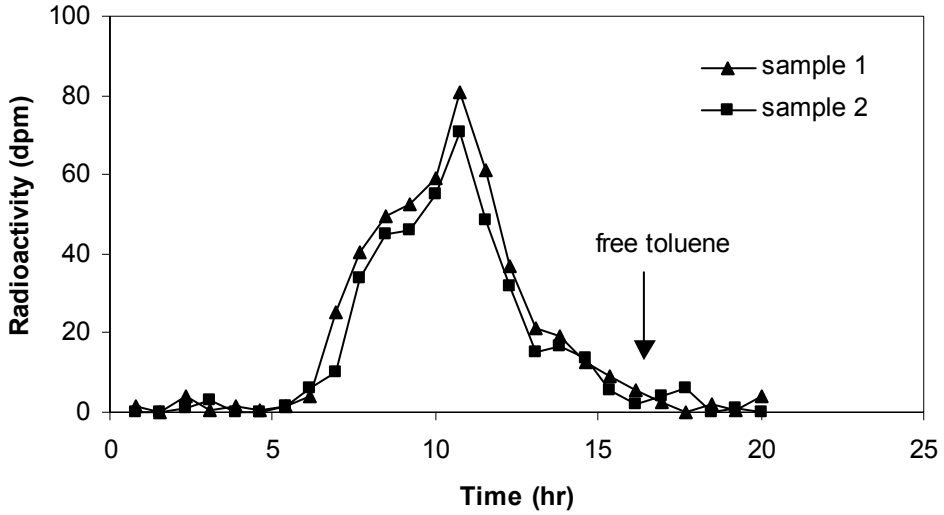

FIGURE 3.6 Analysis of aqueous phase after bioavailability test in methanogenic leachate using HPSEC

Bioavailability tests performed in methanogenic leachate may be complicated because binding of toluene to aqueous-phase organic matter also contributed to the reduction of toluene bioavailability. The extent and rate of binding are determined by factors such as components of leachate and degradation byproducts. The complex nature of the methanogenic leachate and byproducts poses obstacles on differentiation of factors affecting bioavailability. Specifically, it will be difficult to investigate the aging effect on bioavailability of sorbed compounds when other factors are also involved. Moreover, methanogenic leachate exposed to free oxygen is also not an appropriate imitation of municipal landfill leachate. Consequently, bioavailability test will primarily be conducted in growth medium.

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Chapter 4

Results and Discussion

This chapter includes two manuscripts and their supporting information to be submitted for publication. Manuscript 1 summarizes the effect of aging on toluene bioavailability and manuscript 2 summarizes the effect of individual biopolymers on toluene bioavailability.

4.1 The Effect of Aging on the Bioavailability of Toluene Sorbed to Municipal Solid Waste Components

Abstract

Landfills are a unique ecosystem that contains priority pollutants and municipal solid waste with 40-60% organic matter. Interactions of organic contaminants with organic matter in landfills influence their fate and transport. The objective of this study was to evaluate the effects of aging and sorbent decomposition on the bioavailability and fate of toluene sorbed to municipal solid waste (MSW) components. The bioavailability of ^{14}C-toluene aged for 1, 30, and 180 days with individual MSW constituents [office paper, newsprint, model food and yard waste, high density polyethylene (HDPE), and poly(vinyl chloride) (PVC)] was evaluated. Anaerobically degraded sorbents were also tested to evaluate the effect of sorbent decomposition. At the termination of bioavailability tests, sequential solvent and alkaline extractions of sorbents and sorbent combustion were performed to measure the partitioning of ^{14}C that was not coverted to $^{14}CO_2$. The bioavailability of toluene sorbed to MSW components was highest in HDPE, a rubbery polymer; followed by the biopolymers and finally PVC, a glassy polymer. Increased toluene-sorbent contact time reduced the rate of toluene biodegradation for all MSW components except for HDPE. ^{14}C remaining in sorbents at the completion of bioavailability tests was physically sequestered within and/or covalently bound to sorbent organic matter, and this fraction increased with increasing aging time. Up to 20.2% of ^{14}C was associated with humic matter (humic and

fulvic acids, humin). The increasing hydrophobicity of refuse as it decomposes suggests that the concentrations of hydrophobic organic contaminants in leachate should decrease over time.

Introduction

Prior to 1976, there were few restrictions on the co-disposal of industrial waste with municipal solid waste (MSW) in landfills, and as late as 1986 there were an estimated 6000 operating municipal landfills plus thousands of closed facilities that likely accepted some hazardous waste (U.S. EPA, 1988). Many of these sites were constructed without an engineered liner or leachate collection system. Unlined landfills have been shown to result in the contamination of both groundwater and surface water (Reinhard *et al.* 1984; Lesage *et al.* 1990, Cozzarelli *et al*. 2000). Nationally, at least 15% of the sites on the National Priority List of Superfund are municipal landfills that accepted hazardous waste (U.S. EPA, 2003). To protect both ground and surface water resources and design safe and cost-effective remediation strategies, better information on the fate and transport of contaminants in landfills is required.

Alkylbenzenes are among the most frequently detected organic contaminants in landfill leachate (Pohland and Harper 1986; Christenssen *et al*. 1994). While the biodegradation of toluene has been documented in laboratory-scale systems, its frequent detection in methanogenic leachate-contaminated groundwater suggests that its biodegradation in landfills is incomplete (Sanin *et al*. 2000). Biodegradation of hydrophobic organic contaminants (HOCs) is affected by HOC interactions with sorbent organic matter (Schwartz and Scow 1999). Residential refuse is rich in organic matter and contains 30-50% cellulose, 7-12% hemicellulose, and 15-30% lignin. Even in well-decomposed refuse with about 70% of the cellulose and hemicellulose degraded (Ham *et al*. 1993; Wang *et al*.; 1994; Mehta *et al*. 2002), a substantial amount of organic matter remains (Barlaz 1998).

Proposed mechanisms involved in the sequestration of HOCs include adsorption, partitioning, and covalent interactions between metabolites of HOC biodegradation and sorbent organic matter (Hatzinger and Alexander 1995, Dec *et al*. 1997). For nonionic, recalcitrant compounds, adsorption and partitioning are considered the primary mechanisms for HOC sequestration

(Weber and Huang 1996). As the sorbent-HOC contact time (or aging time) increases, HOCs become entrapped in structural voids and the hydrophobic interior of sorbent organic matter. Aging is believed to render contaminants increasingly resistant to biodegradation since these microsites are not accessible to the smallest microorganisms and biodegradation is primarily limited by the slow rate of HOC desorption (Nam and Alexander 1998; White *et al*. 1999). Aging will likely affect long-term contaminant fate and bioavailability in landfills since contaminants are exposed to the buried waste for decades. The metabolites of HOCs biodegradation can also be covalently bound to organic matter through biochemical or abiotic reactions (Bollag *et al*. 1998). As a result of covalent binding, HOCs become an integral part of the sorbent organic matter and are highly resistant to solvent extraction and microbial biodegradation (Dec and Bollag 1988, Achtnich *et al*. 1999).

To date, there has been considerable work on the bioavailability of HOCs in soils and sediments, while there has been little work in the high organic carbon environment of a landfill. This study was initiated to determine the fate of HOCs sorbed to MSW and to investigate the effect of aging time and sorbent decomposition on bioavailability. Toluene was chosen as a model compound because of its frequent occurrence in landfill leachate and its biodegradability in landfills (Christensen *et al*. 1994).

Materials and Methods

Experimental Design. Bioavailability tests were conducted with the major organic components of MSW in pure form to eliminate the complexity and heterogeneity of mixed refuse. The major components of MSW that are expected to exhibit sorptive capacity include paper, food and yard waste, and plastics. Newsprint and office paper were selected to represent the range of paper types in MSW. Newsprint is a mechanical pulp that contains nearly all of the lignin present in wood while office paper is a chemical pulp from which most of the lignin has been removed. Poly(vinyl chloride) (PVC) and high-density polyethylene (HDPE) were tested to represent glassy and rubbery polymers, respectively. Rabbit food that contained both simple and complex carbohydrates as well as proteins in the form of alfalfa, wheat, soy and oat products was used to

represent a combination of food and yard waste. The degradable waste components (office paper, newsprint, rabbit food) were tested in both fresh and anaerobically degraded form to evaluate the effect of sorbent decomposition on toluene bioavailability. Bioavailability tests were conducted under aerobic conditions after preliminary work showed that biodegradation rates obtained under anaerobic conditions were too slow to differentiate controls from tests in the presence of sorbent. Bioavailability was measured after aging of the toluene-sorbent system for 1, 30 and 180 days. Fresh rabbit food was only tested after 1 day of aging because it was difficult to sterilize completely and there were problems with gas accumulation during aging. In addition, fresh rabbit food would not be expected to persist in a landfill for extended time periods because it is readily degradable.

MSW components and chemicals. Rabbit food was obtained in 22.8 kg bags from Manna Pro® Corporation, St. Louis, MO. PVC (42,799-3) and HDPE (18,958-8) were obtained from Sigma-Aldrich (Milwaukee, WI). Newsprint was collected from The News & Observer Recycling Division (News & Observer Publishing Co., Garner, NC). Office paper was collected from the NC State University recycling center. The preparation of degraded sorbents has been described previously (Wu *et al*. 2001). All sorbents except PVC were dried and ground to pass a 1mm screen in a Thomas-Wiley laboratory mill. ^{14}C-toluene (2.8 mCi/mmol, 99% purity) was purchased from Sigma Chemical Co. (St. Louis, MO).

Bioavailability Testing. Prior to aging, sorbents were sterilized in custom-designed ampoules (Fig 4.1) with 2.2 Mrad of γ-irradiation from a ^{60}Co source. The initial quantity of sorbent sterilized was 2 g of PVC and fresh rabbit food; 1.5 g of degraded rabbit food; and 3 g of HDPE, fresh and degraded office paper, and fresh and degraded newsprint. The quantities of both fresh and degraded rabbit food had to be reduced because of their high oxygen demand while the mass of PVC was lower because of its large sorption capacity. After sterilization, 60 mL of growth medium and 20 μL of stock solution, which contained approximately 1.1 μCi of ^{14}C-toluene were added aseptically (only 10 μL of stock solution was spiked in fresh office paper samples because of the low sorption capacity). The stock solution was prepared by mixing 1 mCi of ^{14}C-

toluene with 10 mL of reagent grade toluene to give a final concentration of 120786 dpm/μL. After toluene addition, ampoules were quickly flame sealed to avoid volatilization losses and aged on a shaker table (New Brunswick Innova 2300. Edison, NJ).

After aging, triplicate samples were centrifuged at 3000 rpm for 10 min (Eppendorf 5810, Brinkmann Instruments Inc., Westbury NY). After breaking the flame sealed ampoule at a score mark, the aqueous phase toluene concentration was measured so that duplicate sorbent-free blanks could be prepared at the same liquid phase concentration as aged samples. In the case of office paper and degraded office paper, blanks were prepared from the supernatant that resulted after the sorbents were aged in HOC-free growth medium. This was necessary because the presence of $CaCO_3$ in the sorbent decreased the phosphate buffer capacity during aging and a pH increase was observed during biodegradation when CO_2 was absorbed in the NaOH trap. There was a small loss of toluene during the 30 seconds when the ampoule was broken and the stainless steel Swagelok™ cap was not in place. This loss was quantified by exposing blanks to open air for 30 s and measuring changes in the toluene concentration. Henry's law was used to calculate the loss of toluene in the gaseous phase. Approximately 13.2% of the toluene in the aqueous and gas phases was lost, so the amount of toluene added to the sorbent-containing samples was corrected by this amount. Triplicate samples containing sorbent and two sorbent-free blanks were inoculated with 3 mL of a suspension of *Pseudomonas putida* F1 (*PpF1*) to give 1.5×10^8 cells/mL. After inoculation, the bottle was sealed with a Swagelok™ cap welded with three stainless steel needles (Fig 4.1). Sterile vials were attached to each of the two long needles. One vial contained 2 mL of 2 N NaOH to absorb the evolved $^{14}CO_2$. The second vial contained 1 mL of 30% (v/v) hydrogen peroxide plus 0.5 mL of 0.5% (wt/wt) $FeCl_2$ as a catalyst to promote O_2 production from H_2O_2 to maintain aerobic conditions. The 15-cm needles were sealed with push button syringe valves to prevent volatilization losses. A Tedlar gas bag was attached to the 5-cm needle to prevent a pressure increase from excess oxygen. Samples were incubated at 30±1°C in an incubator shaker (New Brunswick Scientific C24 Classic series, Edison, NJ) operating at 140 rpm. ^{14}C-toluene biodegradation was quantified by the measurement of $^{14}CO_2$ dissolved in the NaOH trap which was sampled at 0.2 – 2 day intervals. Duplicate 0.5-mL NaOH aliquots were

mixed with 6 mL of Ultima Gold scintillation cocktail (Packard BioScience, Meriden, CT). Prior to scintillation counting, samples were refrigerated overnight to reduce chemiluminescence. Fresh NaOH was added after each sampling. To assure that the DO level remained above 3 mg/L, the gas phase oxygen concentration was monitored by a GC equipped with a thermal conductivity detector (Gow Mac Instrument, Bridgewater, NJ). The frequency of H_2O_2 replacement was determined by the oxygen demand of individual sorbents as described below.

Bacterial Strains and Growth Conditions. *PpF1* was grown at 30±1°C in medium containing 4.2215 g KH_2PO_4, 3.368 g K_2HPO_4, 0.05 g $CaCl_2 \cdot 2H_2O$, 0.005 g $FeCl_3$, 0.201 g $MgSO_4 \cdot 7H_2O$, 0.5 g NH_4NO_3, 0.01 g disodium EDTA, 1.43 mg H_3BO_3, 0.32 mg $ZnSO_4 \cdot H_2O$, 0.1 mg $CoCl_2 \cdot 4H_2O$, 0.08 mg $CuSO_4 \cdot H_2O$ and 0.05 mg $Na_2MoO_4 \cdot 2H_2O$ per liter of deionized water. To prepare an inoculum for a bioavailability test, 25 mL of sterile medium was inoculated and *PpF1* was grown in an incubator shaker (150 rpm) with 0.12 mmol toluene as substrate. After 24 hr, 0.5 mL of the culture was transferred into 25 mL of fresh medium. 0.12 mmol toluene was added as substrate and the culture was incubated for an additional 24 hr. The cells were then washed twice with 50 mM pH 7 phosphate buffer and resuspended to an optical density of 0.79 at 600 nm.

Sorbent Mineralization. The oxygen demand of the sorbents was evaluated in preliminary work to ensure that bioavailability tests could be maintained under aerobic conditions. Three gm of γ-irradiated biopolymer sorbents were mixed with 100 mL of sterile growth medium in sterile 250 mL Erlenmeyer flasks sealed with rubber stoppers. *PpF1* was inoculated and samples were incubated at 30±1°C in an incubator shaker with hydrogen peroxide as the oxygen supply. Evolved CO_2 was trapped in 20 mL carbonate free NaOH. Periodically, the NaOH trap was replaced and inorganic carbon was analyzed by a Shimadzu TOC 5000A analyzer (Columbia, MD). The dissolved oxygen concentration in the flask was checked daily using Chemets self-filling DO ampoules (Chemetrics Inc. Calverton, VA). Background CO_2 was measured in an uninoculated flask containing sterile fresh rabbit food.

Measurement of ^{14}C distribution after bioavailability testing. A series of analyses and extractions were conducted to measure the fate of the added ^{14}C-toluene that was not recovered as $^{14}CO_2$. At the completion of a bioavailability test, the sorbent-medium mixture was centrifuged in a 50-mL Teflon™ screw cap centrifuge tube at 3000 rpm for 10 min after which the presence of ^{14}C in the supernatant was measured both before and after filtration through a 0.2 μm cellulose acetate syringe filter (VWR, Atlanta, GA). Preliminary tests showed that there was no significant difference in ^{14}C concentration between direct injection of unfiltered aqueous sample to scintillation cocktail and injection of samples after cell lysis by lysozyme (Table A.1). The difference between filtered and unfiltered samples is ^{14}C associated with particulate matter and biomass. Next, benzyl alcohol (BA) was used to extract sorbed ^{14}C. The sorbent was exposed to 20-25 mL of BA for 200 hr after which the mixture was centrifuged at 3000 rpm for 10 min. Duplicate samples were then removed for scintillation counting. The BA extraction was repeated until less than 0.05% of the total counts added were recovered from the solid. To complete the BA extraction step, the solvent extracted sorbents were washed with deionized water until a separate solvent phase was no longer observed. All BA extracts were combined to report the content of BA extractable ^{14}C. To study the association of ^{14}C-labeled contaminant with humic substances, solvent extracted sorbents were subjected to a series of NaOH extractions. Sorbents were shaken for 200 hr in 20-25 mL 0.1 N NaOH. The sorbent-base mixture was then centrifuged at 3000 rpm for 10 min to obtain a sample of supernatant for scintillation counting. The NaOH extraction was repeated until less than 0.05% of the total counts added were recovered from the solid. All NaOH extracts were combined to report the content of NaOH extractable ^{14}C.

Humic acid (HA) was obtained by acidifying the NaOH extracts to pH 1.5 with 6 N HCl and precipitating for 24 hr at 4°C. The precipitate was collected by centrifugation and then dissolved in 0.1 N NaOH prior to scintillation counting and TOC analysis (Shimadzu TOC 5000A analyzer, Columbia, MD). The supernatant from the pH 1.5 acidification was passed through a 54-mL glass column packed with 50 mL DAX8™ resin ($k'_{0.5r} = 5$) at a flow rate of 12.5 mL/min. Fulvic acid (FA), which was retained on the resin, was eluted with 0.1 N NaOH at 1.5 mL/min

by a peristaltic pump. Nonsorbed effluent was classified as non-humic substances (NH). Both FA and NH fractions were collected for scintillation counting and TOC analysis.

The ^{14}C contained in HA, FA and NH fractions was further characterized by high performance size exclusion chromatography (HPSEC). A Protein Pak 125 column (Waters, Milford, MA) was used in a high performance liquid chromatography (HPLC) system equipped with a UV detector and a Foxy Jr. fraction collector (Isco Inc., Lincoln, Nebraska). The injection volume was 250 μl and detection was performed at 254 nm. The mobile phase was 20 mM pH 7 phosphate buffer (NaH_2PO_4/Na_2HPO_4) with its ionic strength adjusted to 0.1 with NaCl. The eluent flow rate was 1 mL/min. Polystyrene sulfonates of molecular weights from 1.8 to 100 kDa were used as standards. The inner volume of the column was determined using 0.5% acetone. The dead volume was determined with a 0.1% blue dextran solution.

^{14}C that was not extracted from the sorbent was combusted in a Linderberg/Blue tube furnace (Asheville, NC) at 875°C. The exhaust gas was routed to a Barnstead/Thermolyne tube furnace (Dubuque, Iowa) to ensure complete combustion. $^{14}CO_2$ produced was trapped in four successive NaOH traps (15 mL, 2 N) and analyzed by scintillation counting.

Data Analysis. The extent of mineralization of sorbed toluene was calculated from eqn. 1.

$$Extent\ \ of\ mineralization\ \ (\%) = \frac{P_{sorbent} - P_{blank}}{q} \times 100 \qquad (1)$$

Where $P_{sorbent}$ and P_{blank} represent $^{14}CO_2$ (dpm) production from samples containing sorbents and sorbent-free blanks that had the same aqueous-phase toluene concentration as the sorbent-containing samples after aging, and q (dpm) corresponds to the mass of toluene present in a sorbent after aging. Thus, the extent of mineralization at the end of the bioavailability test was calculated based on the conversion of sorbed toluene to $^{14}CO_2$.

The extent of mineralization and the distribution of ^{14}C residue in sorbents after bioavailability tests were compared by an analysis of variance. If the F-statistic from the analysis of variance showed a significant difference ($p < 0.05$), then the pairwise comparison test was used to

determine which samples were different. All statistical analyses were performed by SAS 8.0 software (SAS Institute Inc., Cary, NC).

Results

Sorbent Biodegradation. The fraction of each sorbent degraded by *PpF1* and the maximum oxygen demand are presented in Table 4.1. Mineralization of both fresh and anaerobically degraded office paper and newsprint was below 2%. Based on the size of the inoculum (9×10^{9} cells), a cell mass of 1×10^{-12}g (Brock and Madigan 1991), a cell composition of $C_5H_7O_2N$ and the decay of 20% of the inoculum, this amount of CO_2 can be attributed to biomass decay, indicating that *PpF1* could not hydrolyze much if any lignocellulose. However, the fresh and degraded rabbit food underwent significant mineralization. Rabbit food contained protein and carbohydrates and *Pseudomonads* are known to produce highly active proteolytic enzymes (Pelczar Jr. and Reid 1958).

To calculate the oxygen demand of each sorbent, the biodegradable carbon content in each sorbent was conservatively assumed to be in its most reduced form. The calculation resulted in the maximum possible oxygen demand (Table 4.1). For rabbit food and degraded rabbit food, frequent replacement of H_2O_2 in the initial 72 hr of a bioavailability test was required to maintain aerobic conditions. During bioavailability testing, regular measurement of the gas phase by GC showed that the oxygen concentration was 25-40%, which indicated oxygen was not a limiting factor in toluene biodegradation.

Toluene Bioavailability and the Effect of Sorbent Properties. Mineralization curves for the 1-day aging tests are presented in Fig 4.2 and mineralization curves at other aging times are presented in the Supporting Information (Figs S4.1 and S4.2). One-day aging bioavailability tests were terminated when daily $^{14}CO_2$ production was less than 0.5% of the initially added toluene. Tests were terminated at the same time for all aging periods so that results could be compared. Biodegradation of toluene sorbed to MSW components was bimodal in that it occurred in a fast stage followed by a slow stage. To confirm that the decline in the biodegradation rate was

attributable to substrate availability and not to a loss of microbial activity during prolonged incubation, two samples for each sorbent were reinoculated with *PpF1* after $^{14}CO_2$ production ceased. Reinoculation did not stimulate biodegradation, as evidenced by the insignificant difference between samples with and without freshly added *PpF1* ($p > 0.93$; Fig A.1). Selected samples that were not reinoculated were sacrificed by adding additional ^{14}C-toluene once biodegradation ceased. In all cases, there was a dramatic increase in $^{14}CO_2$ production (Fig A.1), indicating that *PpF1* retained its degradation ability for the duration of bioavailability test.

The mineralization rates observed for toluene sorbed to homogeneous polymers, HDPE and PVC, represent the two extremes in terms of toluene bioavailability (Fig 4.2). Among the tested sorbents, the mineralization rate of toluene in contact with HDPE was the highest. In contrast, the slowest mineralization rate was observed for toluene sorbed to PVC. Blanks without PVC showed a higher initial $^{14}CO_2$ production rate than samples containing PVC, as indicated by the negative portion of the mineralization curve for the first 20 hrs. This result can be explained on the basis that toluene had not reached a sorption equilibrium with PVC after 1 day of aging. [About 250 days were required to reach sorption equilibrium with PVC (Wu *et al*. 2001)]. The continued uptake of toluene by PVC following inoculation likely decreased the liquid phase substrate concentration relative to the blank, resulting in lower $^{14}CO_2$ production rates in samples containing PVC. Initial negative sections of the mineralization curve were not detected after 30 days and 180 days of aging as uptake of toluene by PVC was closer to equilibrium (Figs S4.1 and S4.2 in the Supporting Information).

The mineralization curves for toluene sorbed to biopolymers fell between HDPE and PVC (Fig 4.2). Biopolymer composites are heterogeneous complexes that include cellulose, hemicellulose, lignin, proteins and lipids. The negative portion of the mineralization curve for toluene sorbed to fresh rabbit food in the first 48 hr was likely a result of the preferential biodegradation of rabbit food over toluene (Fig 4.2).

Effect of Aging on Toluene Bioavailability. The percentage of sorbed toluene that was mineralized after aging times of 1, 30 and 180 days is presented in Table 4.2 (at 200 hrs of

incubation) and Table 4.3 (at termination of a bioavailability test). In general, the extent of mineralization decreased as the aging time increased. For fresh and degraded rabbit food, fresh and degraded newsprint and PVC, the aging effect was most significant in the first 30 days. HDPE was the exception to this statement as no statistically significant aging effect was observed ($p > 0.83$).

Several processes contributed to the decrease in toluene bioavailability in office paper and degraded office paper. In addition to aging, a pH change was detected during biodegradation despite the presence of 50 mM phosphate buffer (Table 4.4). During aging, Ca^{2+} released from $CaCO_3$ in office paper likely produced a number of calcium phosphate precipitates such as calcium hydrogen phosphate ($CaHPO_4$), calcium dihydrogen phosphate ($Ca(H_2PO_4)_2$), hydroxyapatite ($Ca_5(PO_4)_3OH$), and β-tricalcium phosphate (β-$Ca_3(PO_4)_2$) (Snoeyink and Jenkins 1980), therefore decreasing phosphate buffer capacity. This precipitation process is slow (Snoeyink and Jenkins 1980), and the loss of buffer capacity increased with increasing aging time. The total phosphate concentration decreased from 3.2 g/L in fresh medium to 2.9 g/L and 1.8 g/L after 1-d and 180-day aging, respectively. The introduction of a NaOH trap in the biodegradation test provided a sink for CO_2, which stimulated $CaCO_3$ dissolution and caused a pH increase. Additional tests confirmed that *PpF1* was inhibited at pH 9 relative to pH 7 (Fig S4.4 in Supporting Information). Thus the pH change likely increased the aging effect as the pH increase inhibited the rate of toluene mineralization.

Distribution of ^{14}C-Toluene at the Completion of Bioavailability Tests. The distribution of ^{14}C-activity in the sorbents upon completion of bioavailability tests is presented in Table 4.5. The total recovery of added ^{14}C-toluene (88.1-101.3%) demonstrated that the experimental setup was suitable for evaluating the bioavailability of a volatile contaminant. The benzyl alcohol extraction recovered all remaining toluene in HDPE and PVC, which was expected because toluene uptake by these plastics is a result of partitioning (HDPE) or a combination of partitioning and adsorption (PVC). The quantity of ^{14}C extracted from HDPE was relatively constant for the three aging times. In contrast, 17% more ^{14}C was extracted from PVC when the aging time increased from 1 to 30 days ($p < 0.001$) and an additional increase was measured

between 30 and 180 days ($p < 0.001$). This increase in solvent extractable ^{14}C with aging time mirrors the findings of toluene bioavailability. More toluene diffused into the physically remote sites of PVC as the aging time increased. Consequently, more time was required for the toluene molecules to diffuse out of the sorbent and become bioavailable to microorganisms.

Biopolymer sorbents contain humic substances and toluene associated with humic matter would not be extracted with BA. To determine the change in the distribution of non-bioavailable ^{14}C, BA extractable ^{14}C was normalized based on the total ^{14}C that remained in the sorbents after bioavailability testing (Table 4.6). The BA extractable ^{14}C from all biopolymers decreased with increasing aging time, indicating the transformation of the remaining ^{14}C from a labile to a more strongly bound fraction.

^{14}C Association with Humic Substances. ^{14}C that remained after extraction with BA was extracted with NaOH and classified as HA, FA, NH and humin based on operational definitions (Thurman and Malcolm 1981). HA is defined by its solubility in dilute alkaline solutions and by precipite formation at $pH < 2$, while FA is defined by its solubility in acids and bases. Following precipitation of HA, the FA and NH fractions were separated using a DAX8™ resin. NH substances were not retained by the DAX8™ resin and are likely comprised amino acids and organic acids synthesized by living organisms (Stevenson 1994). Humin is humic matter that is insoluble in dilute acids and bases. Radioactivity was detected in all humic fractions except for HA fractions in fresh and degraded office paper (1, 30 and 180-day aging) and the FA and NH fractions in fresh office paper (1-day aging) (Table 4.5).

To determine the molecular weight fraction that contains ^{14}C, HA, NH and FA extracted from MSW sorbents were fractionated by HPSEC. A typical chromatogram illustrating HA extracted from rabbit food is shown in Fig 4.3. The retention time of toluene was determined by monitoring the absorbance of a sample containing toluene only. The HPSEC results indicate that the ^{14}C in the HA fraction extracted from rabbit food was associated with organic matter with a molecular size greater than toluene. Chromatograms of HA, FA, and NH fractions extracted from other sorbents all indicated the presence of ^{14}C in relatively high molecular weight fractions (Fig

S4.3 in Supporting Information). Furthermore, the absence of free toluene suggests that ^{14}C in NaOH extractable fractions existed only in a bound form (either toluene was strongly associated with the organic matter or toluene metabolites were covalently bound to the organic matter).

The FA fraction accounted for 76-99% of the non-purgable organic carbon (NPOC) in NaOH extract (Table 4.7). However, HA retained 3.7-24.3 times more ^{14}C than FA when normalized for organic carbon (Table 4.8). Similarly, the NH fraction, which likely contains biochemical compounds synthesized by microorganisms, contained 8.1-25.9 times more ^{14}C than FA.

With the exception of fresh office paper, the percentage of ^{14}C in the humin fraction increased with aging time (Table 4.9), further illustrating the importance of aging on toluene sequestration.

Discussion

Synthetic Polymers. HDPE and PVC are synthetic polymers that consist of repeating ethylene and vinyl chloride monomers, respectively. HDPE and PVC behaved differently with respect to sorption and bioavailability. The different behavior can be attributed to the dissimilarities in their polymeric structures and particle size. At the temperature at which the bioavailability tests were conducted, HDPE is a rubbery polymer while PVC is a glassy polymer. Rubbery polymers have an expanded, flexible structure while glassy polymers have a more rigid, condensed structure (Xing *et al*. 1996). Both rubbery and glassy polymers contain regions into which HOCs can partition. In addition, glassy polymers contain hole sites, where HOCs can adsorb. HOC diffusion through glassy polymers are lower than through rubbery polymers because the glassy polymer structure is more rigid and because adsorption energies of hole sites are large (Weber *et al*. 1992). Abiotic desorption tests showed that up to 99% of the sorbed toluene was released from HDPE within 70 hours. However, toluene sorbed to PVC was released more slowly and only 33% was released within the same time period. Diffusion of toluene through homogeneous polymers can be described by Fick's second law of diffusion, which dictates that the desorption rate of toluene is positively related to the diffusion coefficient and inversely related to the square of particle diameter. The diameter of HDPE (500 μm) was about 3.6 times larger than that of PVC (140 μm) while the diffusion coefficient of toluene in HDPE ($\sim 10^{-9}$ cm^2/s) (Rogers *et al*.

1960) is several orders of magnitude larger than in PVC (10^{-13} -10^{-14} cm^2/s) (Berens 1989). Consequently, the more rapid degradation of toluene sorbed to HDPE can be explained by the more rapid release of toluene from HDPE particles. As the aging time increased, toluene molecules diffused further into the rigid organic matter and hole sites of PVC. Therefore, the diffusion path length increased as aging time increased, which explains why toluene release rates and thus $^{14}CO_2$ production decreased with increasing aging time on PVC. In HDPE, however, toluene was present only in expanded dissolution sites, from which desorption rates are fast. Therefore, the toluene release rate from HDPE was not significantly affected by toluene-sorbent contact time. The ^{14}C that remained in HDPE and PVC after bioavailability test was readily extractable by BA. The relatively constant ^{14}C in BA extracts of HDPE with aging and the time-dependent increase of ^{14}C in BA extracts of PVC are consistent with the finding that increased aging time significantly reduced the bioavailability of toluene sorbed to PVC while no effect was observed with HDPE (Table 4.2).

Biopolymers. The mineralization rate of toluene sorbed to both fresh and degraded biopolymers fell between those for the two synthetic polymers (Fig 4.2). This could be attributed to the lower sorption capacity of biopolymers for toluene than that of HDPE and consequently less mass of substrate released from biopolymers. In addition, preferential degradation of organic matter in biopolymers, e.g. fresh rabbit food may also decrease the biodegradation rate of sorbed toluene. Because of presence of rapid desorption fraction, biodegradation rate of toluene sorbed to biopolymers is faster than that to PVC.

The extent of mineralization followed the similar trend in the initial 200 hrs of incubation. However, this may not be true if the incubation time is extended. Despite the slow release rate, toluene sorbed to synthetic polymers would be completely available to microorganisms after sufficient incubation because of the absence of humic substances. For biopolymers, complete mineralization of the sorbed toluene is not expected because of the association of ^{14}C with humic substances and the poor biodegradability of bound ^{14}C.

Relative to the 1-d aging time, the bioavailability of toluene sorbed to biopolymers was reduced

by 11-22% and 12-29% after 30 and 180 days of aging, respectively. The aging effect can be explained by the continued diffusion of toluene molecules into condensed organic matter and the formation of stronger associations between ^{14}C and sorbent organic matter. These results were also confirmed by abiotic desorption data, which showed a decrease in the fast desorbing fraction and an increase of slow desorbing fraction with increasing aging time (Wu 2002). Although the bioavailability of toluene sorbed to fresh and degraded office paper appeared to decrease with increasing aging time, this result was at least in part a result of a pH increase that occurred during the biodegradation step, and this pH increase decreased the rate of toluene mineralization. As explained above, the magnitude of the pH rise increased as the aging time became longer (Table 4.4). Furthermore, ingredients in fresh office paper may have had an inhibitory effect on *PpF1* (Fig S4.5 in Supporting Information). To fully understand this issue, additional studies are needed to quantify the effect of the chemicals involved.

The percentage of solvent extractable radioactivity based on total sorbent-retained radioactivity decreased with increasing aging time. Although it is suggested that HOCs sequestered in remote microsites of organic matter can be removed by solvent extraction (Wershaw 1993; Dec *et al*. 1997), the definition of solvent extractable residue is not as clear as suggested by the terminology. For example, Dec *et al*. (1997) suggested that the fraction of ^{14}C that cannot be solvent extracted is sorbed to humin fraction without being chemically modified. Guthrie *et al*. (1999) reported that pyrene that is bound to the humin fraction by van der Waals forces and hydrogen bonding could not be removed by stringent solvent extraction methods. Similarly, using solvent extraction to predict bioavailability of HOCs is a poorly defined process since it is strongly affected by the species of sorbate, type of sorbent and solvent, and extraction techniques (Alexander 2000; Reddy *et al*. 2000; Tang *et al*. 2002, Johnson *et al*. 2002). An organic solvent such as BA could swell the sorbent organic matrix and open diffusive pathways in those regions of the sorbent organic matter from which alkylbenzene partially desorbed. Also, ^{14}C associated with lipophilic extractives could also be extracted when lipophilic extractives dissolve in BA. However, it is not clear that the BA extractable fraction represents the ultimate toluene bioavailability.

The presence of ^{14}C in the humic substances of biopolymers was not surprising as humic substances are formed by the chemical and biological transformation of lignocellulosic materials (Wershaw 1993). Both the precursors, lignin in particular, and humic substances contain reactive functional groups that can act as binding sites for HOCs (Stevenson 1994). The type of association between pollutants and humic substances could include ester bonds formed by condensation reactions (Richnow *et al*. 1993, Käcker *et al*. 2002) as well as ether and carbon-carbon bonds formed by enzyme-mediated oxidative polymerization (Richnow *et al*. 1997). Similar transformations were also expected for toluene because microbial degradation of toluene results in hydroxylated aromatic compounds that are chemically more reactive than the parent compound. These compounds have been detected in growth medium (Dagley *et al*. 1964, Gibson *et al*. 1968, Yu *et al*. 2001). Metabolites such as *cis,cis*-2-hydroxy-6-oxohepta-2,4-dienoate and 4-hydroxy-2-oxo-valerate bear carboxylic groups which may be capable of forming ester bonds with humic substances. Aromatic alcohols such as 3-methylcatechol were suggested to bind to humic substances through enzymatic cross-coupling (Richnow *et al*. 1997).

Treatment of biopolymers with NaOH causes the disruption of H-bonding in cellulose and hemicellulose, breakage of ester linkages, and deprotonation of acidic carboxylic and phenolic groups (Akin *et al*. 1992, Simpson *et al*. 2003). As a result, swelling of cellulose and the partial solubilization of protein, hemicellulose and lignin are expected (Jackson 1977, Scalbert and Monties 1986, Tomotake *et al*. 2002). In the case of office paper, sizing agents such as alkenyl succinic anhydrides (ASA) and alkyl ketene dimers (AKD) are bound to cellulose through a β-keto ester linkage and would be released during NaOH treatment (ASA and AKD are neutral pH sizing agents which are applied to paper with large $CaCO_3$ usage) (Roberts 1996). This is a likely explanation for the presence of ^{14}C in the NaOH extract of fresh and degraded office paper. In the case of fresh rabbit food, the ^{14}C in humic substances was primarily attributed to the partial dissolution of lignin and/or ^{14}C that was covalently linked to lignin. Alkali dissolved lignin has a higher degree of polymerization and it may also contain co-extracted polysaccharides, which explains the association of ^{14}C with substances of large molecular weight (Fig 4.3, Fig S4.3 in Supporting Information) (Xiao *et al*. 2001, Durot *et al*. 2003). In fresh newsprint, besides lignin, NaOH could also remove some wood extractives like phenolic acids (Akin *et al*. 1992, Martens

2000). Phenolic acids such as ferulic acid and syringaldehyde were reported to be very reactive during oxidative coupling reactions where covalent bonds are formed between metabolites of organic compounds and humic substances.

Compared to NaOH extracts of fresh biopolymers, the NaOH extract of degraded biopolymers contains both fresh and humified lignocelluloses. Except for humic acids extracted from degraded office paper, ^{14}C activity was detected in all fractions of humic matter. The lack of humic acids in degraded office paper may be attributed to its extremely lower lignin content as humic acids are proposed to result from the condensation of modified lignin with protein (Bollag *et al*. 1998). Humic acids are rich in phenolic groups (Weber *et al*. 2000), compared to which fulvic acids are lower in aromaticity because fulvic acids possess a significant concentration of oxygen-containing carboxyl and alcohol functional groups (Hatcher and Spiker 1983, Aochi and Farmer 1997). Because of their higher polarity and lower aromaticity, fulvic acids bound less ^{14}C-toluene than humic acids (Table 4.8).

Except for fresh office paper, the combustion data showed that ^{14}C associated with the humin fraction of biopolymer composites increased with aging. The humin structure is dominated by undegraded and slightly degraded biopolymers, such as lignin and polysaccharides (Hatcher *et al*. 1985). Humin is believed to represent the glassy domain of organic matter because it has a more condensed structure than humic acids which may restrict the diffusion of HOCs and it appears to be enriched in subnanometer-size voids that might provide specific sorption sites for organic compounds (White *et al*. 1999, de Jonge *et al*. 2000). During aging, more toluene molecules were trapped in humin through the combination of diffusion into micropores and partition into organic matter, resulting in enrichment of radioactivity over time.

Environmental Significance. Although bioavailability tests were conducted under aerobic condition, the test results can be extended to the landfill ecosystem, which is anaerobic. Because microorganisms are size-excluded from a sorbent matrix, it is generally believed that all biodegradation occurrs in the dissolved phase (Ogram *et al*. 1985; Johnson *et al*. 1999) and bioavailability is controlled by the rate of contaminants desorption (Rockne *et al*. 2002).

Although the biodegradability of soil-sorbed compounds has been reported (Racke and Lichtenstein 1985; Guerin and Boyd 1997), no direct evidence was presented to indicate that sorbed compounds were degraded without being released to the aqueous phase or sorbent surface.

Estimating the change of HOC concentrations in landfill leachate over time is difficult because the increase of sorbent hydrophobicity is counterbalanced by the loss of sorbent organic matter. Reinhart *et al*. (1990) reported that the increases in sorbent hydrophobicity as a result of biopolymer degradation had little effect on K_{oc} for sorption of HOCs to relatively nonpolar refuse, suggesting that landfill leachate HOC concentrations would increase over time due to the loss of sorbent organic matter. For a landfill where paper waste dominates, however, given the fact that the preferentially decomposed cellulose and hemicellulose have poor sorptive capacity for HOCs and the resynthesized lipids and proteins have higher affinity for HOCs, it would suggest that HOC concentrations in leachate should decrease with time.

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4.2 Tables and Figures for Manuscript 1

FIGURE 4.1 Bioavailability Reactor.

Same as FIGURE 3.1.

TABLE 4.1 Degradation of Sorbents by *PpF1*

sorbent	sorbent mass (g)	f_{oc} [a]	% C degraded [b]	Maximum daily O_2 demand (mg) [c]
fresh office paper	3	0.373	1.18	24.67
degraded office paper	3	0.278	1.95	37.01
fresh newsprint	3	0.451	0.81	10.55
degraded newsprint	3	0.455	0.78	22.33
fresh rabbit food	2	0.423	34.53	547.51
degraded rabbit food	1.5	0.329	6.89	46.85

[a] Fraction of organic carbon (Wu *et al*. 2001). [b] The ratio of cumulative inorganic carbon produced to the organic carbon content of each sorbent. Values are averages of triplicate samples. [c] See text for calculation.

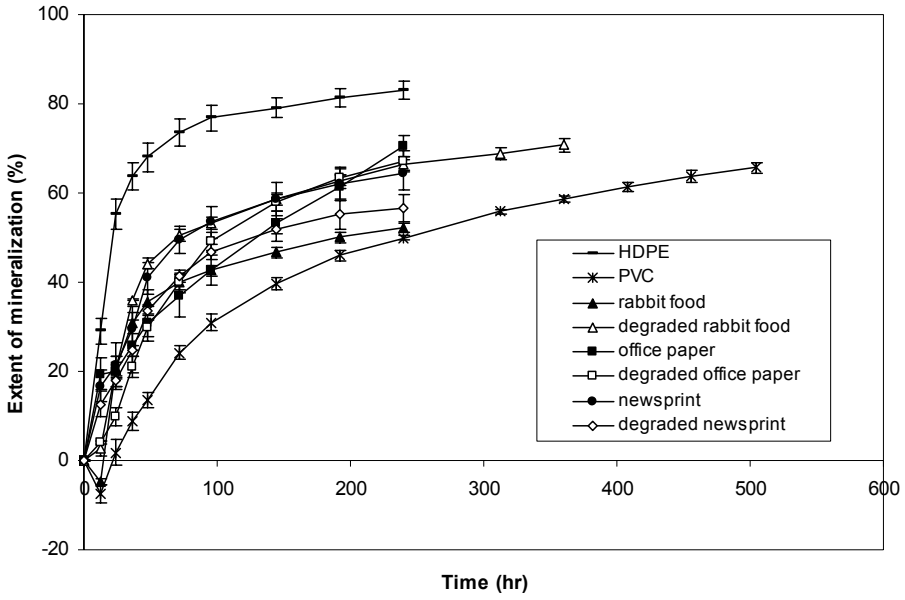


FIGURE 4.2 Bioavailability of Toluene Sorbed to MSW Components after 1-d aging as calculated from eqn. 1.

Data are averages of three replicates. Error bars represent standard deviations. Bioavailability tests were stopped when daily $^{14}CO_2$ increase was below 0.5% of total toluene added.

TABLE 4.2 Mineralization of Toluene Sorbed to MSW Components at 200 hrs (%) [a]

sorbent	1-d aging	30-d aging	180-d aging
HDPE	81.3 (3.1) A[b]	80.6 (1.3) A	78.7 (1.2) A
PVC	46.0 (1.3) A	37.9 (1.1) B	34.2 (0.3) C
fresh office paper	61.2 (2.7) A	0 [c]	0 [c]
degraded office paper	63.2 (2.2) A	38.8 (1.9) B	32.9 (2.2) C
fresh newsprint	61.1 (3.7) A	44.4 (2.7) B	43.7 (1.6) B
degraded newsprint	56.7 (3.1) A	46.1 (1.3) B	45.2 (1.5) B
fresh rabbit food	50.1 (1.1)	na [d]	na [d]
degraded rabbit food	62.9 (1.2) A	53.9 (3.7) B	43.8 (3.0) C

[a] Data are presented as the percent of the sorbed ^{14}C-toluene that was converted to $^{14}CO_2$ as in eqn 1. Values are averages of 3 or 4 samples. The values in parentheses are the standard deviations. [b] Values in rows for the same sorbent followed by the same letter are not statistically different ($p > 0.05$). [c] Mineralization in sorbent containing samples did not exceed that in the respective blanks. [d] Not analyzed.

TABLE 4.3 Mineralization of Toluene Sorbed to MSW Components (%) [a]

sorbent	1-d aging	30-d aging	180-d aging
HDPE	82.9 (1.9) A[b]	81.8 (1.3) A	79.9 (1.1) A
PVC	65.6 (1.3) A	57.8 (0.9) B	52.2 (0.6) C
fresh office paper	66.1 (2.2) A	0 [c]	0 [c]
degraded office paper	67.1 (2.5) A	45.4 (0.6) B	37.8 (2.1) C
fresh newsprint	64.3 (3.7) A	47.1 (2.5) B	46.0 (2.1) B
degraded newsprint	60.4 (3.3) A	49.5 (1.2) B	48.4 (1.5) B
fresh rabbit food	52.1 (1.0) A	na [d]	na [d]
degraded rabbit food	70.7 (1.6) A	59.9 (3.4) B	52.8 (2.8) C

[a] Data are presented as the percent of the sorbed ^{14}C-toluene that was converted to $^{14}CO_2$ as in eqn. 1. Values are averages of 3 or 4 replicates. The values in parentheses are the standard deviations. [b] Values in rows for the same sorbent followed by the same letter are not statistically different ($p > 0.05$). [c] Mineralization in solid containing samples did not exceed that in their respective blanks. [d] Not analyzed.

TABLE 4.4 pH at the Termination of Bioavailability Tests for Selected Sorbents

sorbent	$CaCO_3$ in sorbent (g)	1-d aging	30-d aging	180-d aging
fresh office paper	0.268	7.01	8.65	9.10
degraded office paper	0.537	7.50	8.68	9.32
fresh newsprint	0.006	7.59	6.94	6.96
degraded newsprint	0.016	na [a]	6.84	6.92
degraded rabbit food	0.060	na [a]	7.24	7.33

[a] Not analyzed.

TABLE 4.5 Distribution of Radioactivity (%)

sorbent	aging time (d)	sorbed [b]	$^{14}CO_2$ [a]	particulate and cell mass [a,c]	non-volatile ^{14}C in liquid [a]	solvent extractable [a,d]	humic substances				total recovery [a,g]
							HA	FA	NH	humin	
PVC	1	53.7 (2.1)	68.5 (0.7)	9.1 (1.9)	2.0 (0.1)	19.3 (0.3)	na [e]				98.8 (1.9)
PVC	30	89.7 (0.5)	56.4 (0.8)	5.6 (0.1)	1.7 (0.2)	36.2 (0.7)	na				99.9 (1.3)
PVC	180	91.8 (0.2)	52.3 (0.6)	5.6 (0.1)	2.3 (0.4)	37.4 (0.7)	na				97.6 (0.7)
HDPE	1	75.9 (0.7)	78.4 (0.1)	16.4 (0.6)	2.6 (0.1)	3.4 (0.1)	na				100.9 (0.4)
HDPE	30	75.9 (1.1)	75.7 (1.0)	19.5 (1.7)	3.4 (0.1)	2.8 (0.1)	na				101.3 (2.2)
HDPE	180	75.9 (0.6)	72.5 (1.0)	20.8 (3.0)	4.4 (0.1)	2.5 (0.2)	na				100.3 (1.8)
fresh office paper	1	16.3 (1.8)	66.1 (0.4)	10.0 (0.7)	2.5 (0.2)	2.4 (0.3)	nd [f]	nd	nd	7.8 (0.9)	88.8 (1.2)
fresh office paper	30	23.5 (2.5)	49.3 (0.8)	10.4 (0.0)	5.9 (0.1)	3.3 (0.3)	nd	4.8 (0.0)	0.6 (0.0)	13.8 (0.4)	88.1 (1.5)
fresh office paper	180	26.7 (1.7)	43.6 (0.8)	11.3 (2.3)	16.2 (3.3)	1.2 (0.6)	nd	2.9 (0.5)	1.2 (0.5)	14.7 (1.2)	90.4 (1.8)
degraded office paper	1	37.8 (0.2)	71.0 (1.0)	7.9 (0.3)	2.3 (0.0)	4.9 (0.4)	nd	1.5 (0.3)	0.6 (0.1)	5.8 (0.2)	94.4 (1.2)
degraded office paper	30	41.4 (0.9)	61.2 (0.3)	7.1 (0.3)	9.4 (0.2)	5.8 (0.4)	nd	2.1 (0.3)	1.7 (0.4)	8.8 (0.9)	91.6 (0.6)
degraded office paper	180	42.1 (0.4)	54.3 (0.4)	4.7 (0.2)	16.6 (0.0)	5.0 (0.3)	nd	0.9 (0.0)	1.3 (0.2)	10.4 (0.3)	93.4 (1.0)
fresh newsprint	1	42.0 (0.3)	71.8 (0.4)	6.1 (0.6)	2.3 (0.0)	3.6 (0.2)	1.7 (0.0)	1.4 (0.1)	1.2 (0.1)	3.7 (0.6)	92.2 (1.0)
fresh newsprint	30	46.3 (0.8)	63.5 (1.1)	5.6 (0.4)	5.1 (0.1)	4.0 (0.3)	2.6 (0.5)	2.0 (0.1)	1.1 (0.1)	6.5 (0.7)	90.3 (1.1)
fresh newsprint	180	46.6 (0.9)	61.1 (0.3)	5.9 (0.1)	8.1 (0.0)	2.7 (0.3)	2.2 (0.0)	2.5 (0.1)	1.5 (0.3)	8.6 (1.2)	92.6 (1.1)
degraded newsprint	1	45.9 (0.7)	67.4 (1.6)	9.2 (0.4)	3.2 (0.2)	6.6 (0.3)	2.9 (0.5)	1.3 (0.1)	0.5 (0.1)	6.0 (0.3)	97.1 (0.9)
degraded newsprint	30	48.2 (1.1)	62.4 (1.3)	9.4 (0.8)	3.2 (0.1)	7.0 (0.3)	3.0 (0.9)	1.8 (1.1)	0.4 (0.0)	7.0 (0.5)	94.0 (2.9)
degraded newsprint	180	49.5 (1.8)	63.0 (0.0)	6.3 (0.7)	2.7 (0.1)	8.3 (0.5)	2.1 (0.1)	2.7 (0.1)	1.3 (0.1)	10.2 (0.6)	93.5 (0.7)
fresh rabbit food	1	41.6 (1.3)	62.4 (0.5)	10.1 (0.5)	4.4 (0.7)	5.6 (0.6)	2.4 (0.2)	1.8 (0.2)	2.4 (0.2)	1.0 (0.2)	91.2 (1.0)
degraded rabbit food	1	39.6 (0.7)	70.8 (0.7)	11.8 (0.6)	4.5 (0.1)	4.1 (0.1)	0.3 (0.0)	1.1 (0.0)	1.1 (0.1)	0.7 (0.1)	94.4 (0.4)
degraded rabbit food	30	39.7 (0.2)	65.7 (1.4)	10.9 (0.2)	4.8 (0.2)	4.5 (0.2)	2.5 (0.0)	0.8 (0.1)	0.9 (0.1)	0.9 (0.0)	91.1 (1.2)
degraded rabbit food	180	39.8 (0.5)	63.4 (0.4)	11.7 (0.3)	6.2 (0.7)	4.6 (0.2)	0.7 (0.0)	2.3 (0.8)	2.1 (0.3)	1.3 (0.1)	92.2 (0.5)

[a] Data are presented as a percentage of the total amount of toluene added initially corrected for volatile loss. Data are the averages of three ampoules for each sorbent and the standard deviation is presented parenthetically. [b] ^{14}C in solid phase before inoculation. Data were presented without correction for volatile loss. [c] Includes biomass and particulates. [d] Solvent is benzyl alcohol.. [e] Not analyzed. [f] Radioactivity was not detected. The detection limit varies with the amount of toluene added initially and ranged from 0.001-0.002%. [g] Total recovery includes ^{14}C recovered as $^{14}CO_2$, particulate and biomass, non-volatile, solvent extractable and humic substances associated ^{14}C.

TABLE 4.6 Solvent Extraction of Radioactivity by Benzyl Alcohol (%) [a]

sorbent	1-d aging	30-d aging	180-d aging
fresh office paper	25.8 (1.4) A	14.1 (0.6) B	6.01 (2.9) C
degraded office paper	38.5 (0.5) A	31.4 (2.3) B	28.2 (0.3) C
fresh newsprint	31.5 (1.6) A	25.2 (1.5) B	15.3 (0.1) C
degraded newsprint	38.9 (2.4) A	36.5 (1.1) B	33.9 (1.0) C
fresh rabbit food	42.4 (4.7)	na [c]	na [c]
degraded rabbit food	55.5 (0.9) A	46.6 (3.0) B	41.4 (2.7) C

[a] Values are the percentage of benzyl alcohol extracted ^{14}C based on the total activity remaining on a sorbent after bioavailability testing was complete. Data in parentheses are the standard deviations. [b] Values in rows for the same sorbent followed by the same letter are not statistically different ($p > 0.05$). [c] Not analyzed.

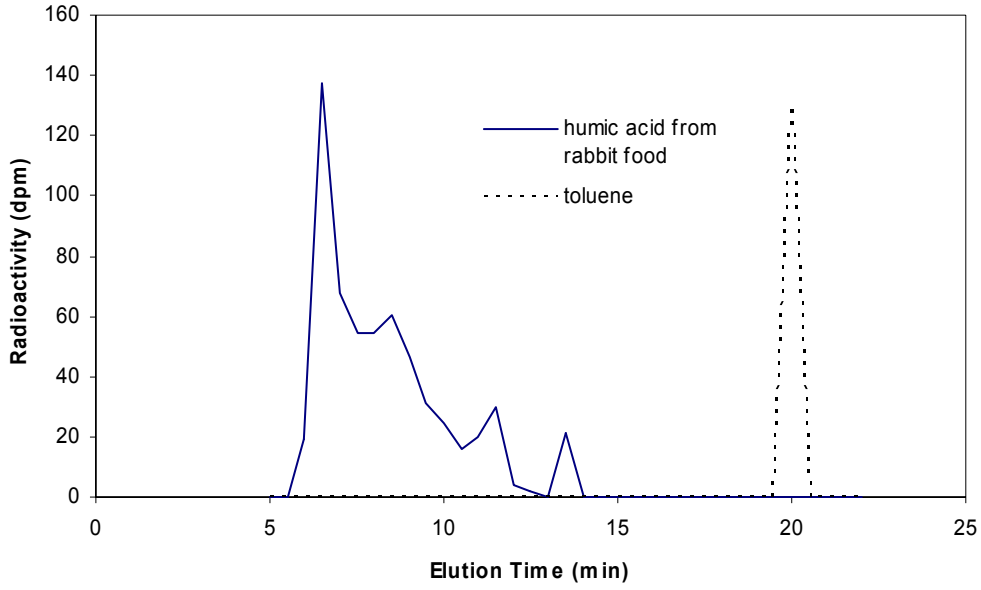


FIGURE 4.3 HPSEC chromatogram of humic acid extracted from rabbit food.

The humic acid fraction was adjusted to pH 6. Eluent was collected and analyzed by scintillation counting.

TABLE 4.7 Distribution of Non Purgable Organic Carbon (NPOC) in Humic Matter (%) [a]

	1-d aging			30-d aging			180-d aging		
	HA[b]	NH[b]	FA[b]	HA	NH	FA	HA	NH	FA
fresh office paper	nd[c]	nd	nd	nd	1.3	98.7	nd	1.4	98.6
fresh newsprint	4.4	5.0	90.6	13.3	3.2	83.5	3.8	1.3	94.9
degraded newsprint	21.1	2.4	76.5	15.2	2.4	82.4	10.1	1.4	88.5
fresh rabbit food	7.6	4.5	87.9	na[d]	na	na	na	na	na
degraded rabbit food	2.1	5.3	93.4	11.6	4.1	84.4	8.9	2.4	89.7

[a] Values are the percentage of NPOC in one fraction based on the total NPOC in the NaOH extract. Data are averages of triplicate samples. CV ≤ 26.6%. [b] HA – humic acid, NH – non-humic matter, FA – fulvic acid [c] Radioactivity was not detected. [d] Not analyzed.

TABLE 4.8 Association of Radioactivity by Humic Substances

Sorbent	[^{14}C]/NPOC (dpm/mg)[a]								
	1-d aging			30-d aging			180-d aging		
	HA	NH	FA	HA	NH	FA	HA	NH	FA
fresh office paper	nd[b]	nd	nd	nd	0.19	0.02	nd	0.24	0.01
degraded office paper	nd	0.20	0.01	nd	0.30	0.03	nd	1.43	0.02
fresh newsprint	0.37	0.24	0.02	0.15	0.27	0.02	0.13	0.68	0.02
degraded newsprint	0.12	0.18	0.02	0.20	0.18	0.02	0.16	0.72	0.02
fresh rabbit food	0.27	0.46	0.02	na[c]	na	na	na	na	na
degraded rabbit food	0.31	0.39	0.02	0.40	0.40	0.02	0.15	1.61	0.04

[a] Values are averages of duplicate or triplicate samples. CV ≤ 28.2%. [b] Radioactivity was not detected. [c] Not analyzed.

TABLE 4.9 Association of Radioactivity with Humin Fraction (%) [a]

sorbent	1-d aging	30-d aging	180-d aging
fresh office paper	80.4 (2.0) A[b]	61.1 (0.8) B	73.8 (0.4) A
degraded office paper	45.2 (0.1) A	47.9 (1.9) B	59.1 (0.3) C
fresh newsprint	31.6 (3.3) A	39.9 (1.2) B	49.4 (1.8) C
degraded newsprint	34.2 (0.8) A	36.7 (3.6) B	41.1 (0.9) C
fresh rabbit food	7.4 (1.6) A	na[c]	na[c]
degraded rabbit food	9.5 (1.5) A	9.4 (0.3) A	10.8 (0.2) A

[a] Data are percentage of radioactivity associated with humin fraction based on the total activity remaining on the sorbents after bioavailability testing. Values are averages of triplicate samples. The values in parentheses are the standard deviations. [b] Values in rows for the same sorbent followed by the same letter are not statistically different ($p > 0.05$). [c] not analyzed.

4.3 Supporting Information for Manuscript 1

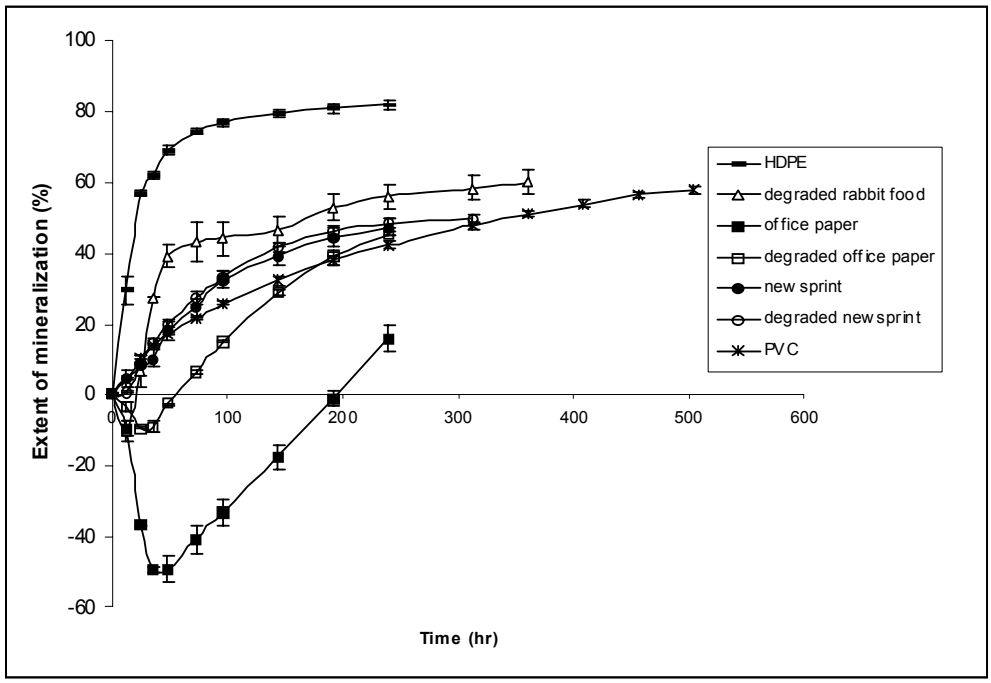


FIGURE S4.1 Bioavailability of Toluene Sorbed to MSW Component after 30-d aging.

Data are averages of three replicates. Error bars represent standard deviations.

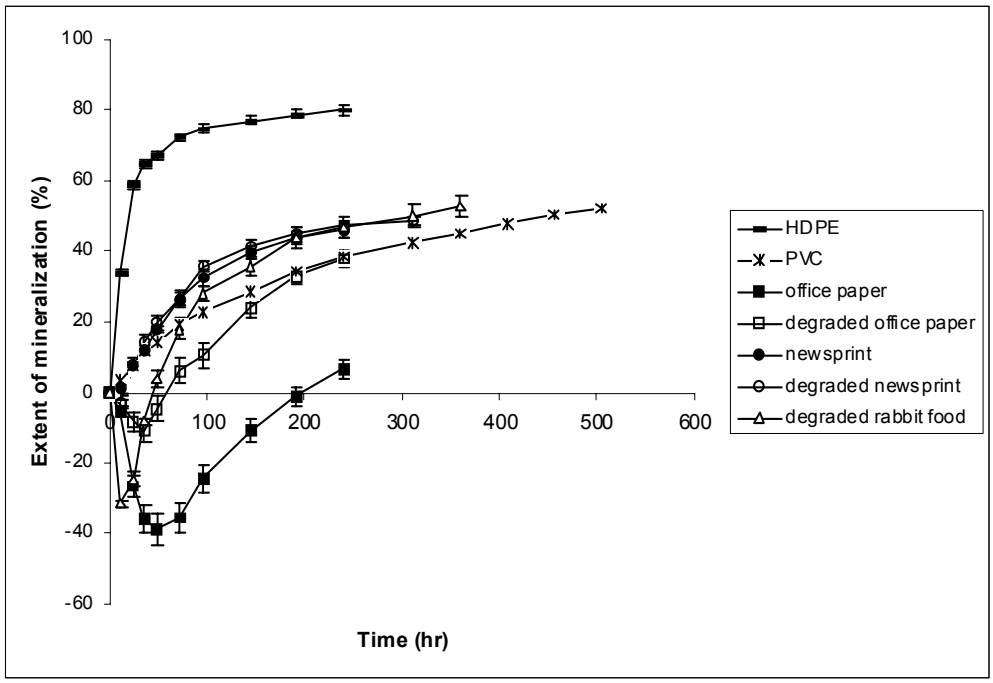
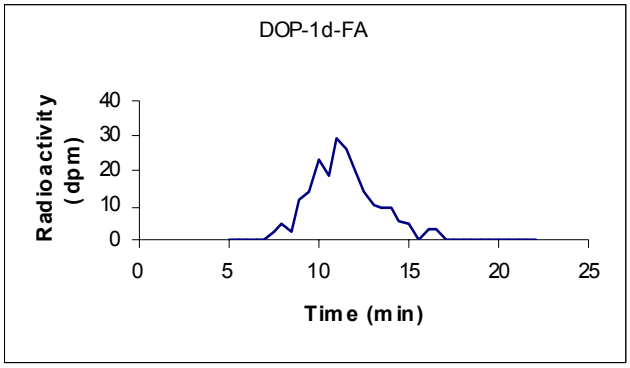


FIGURE S4.2 Bioavailability of Toluene Sorbed to MSW Component after 180-d aging.

Data are averages of three replicates. Error bars represent standard deviations.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Radioactivity (dpm)
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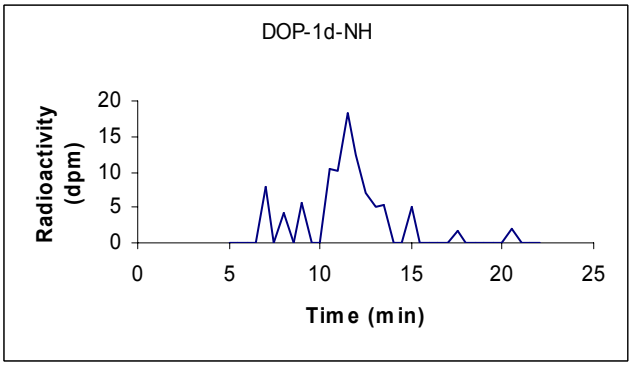
10
20
30
40
0
5
10
15
20
25
Time (min)


DOP-1d-NH
Radioactivity (dpm)
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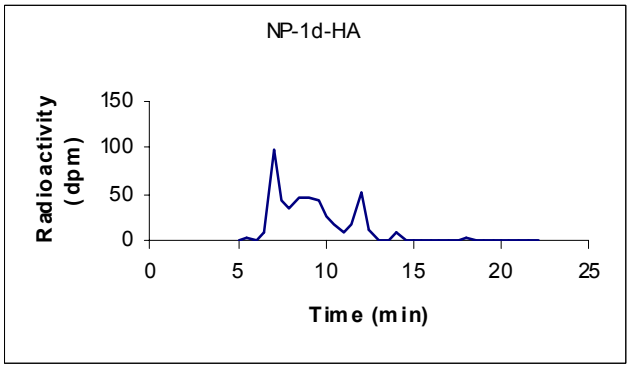
5
10
15
20
0
5
10
15
20
25
Time (min)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Radioactivity (dpm)
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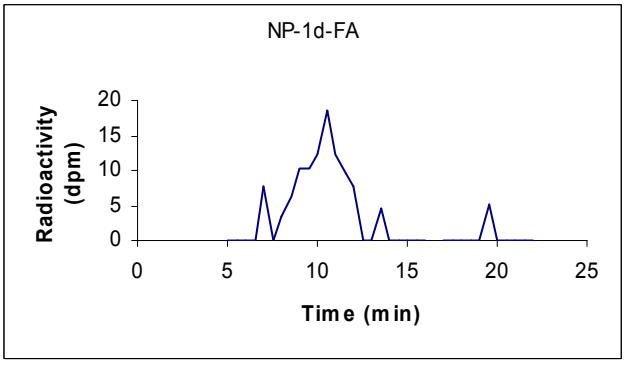
50
100
150
0
5
10
15
20
25
Time (min)


NP-1d-FA
Radioactivity (dpm)
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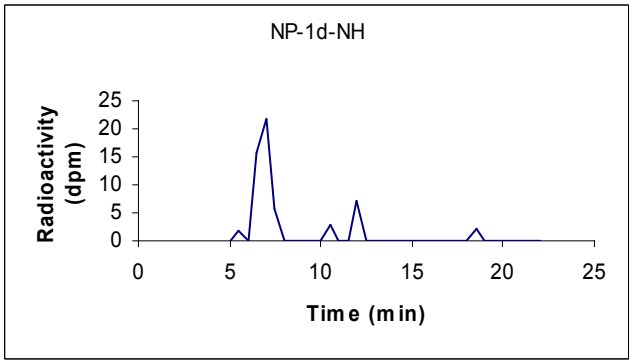
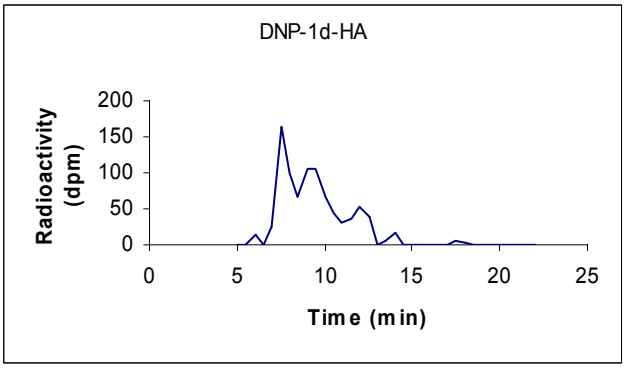
5
10
15
20
0
5
10
15
20
25
Time (min)


NP-1d-NH
Radioactivity (dpm)
0
5
10
15
20
25
0
5
10
15
20
25
Time (min)


DNP-1d-HA
Radioactivity (dpm)
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100
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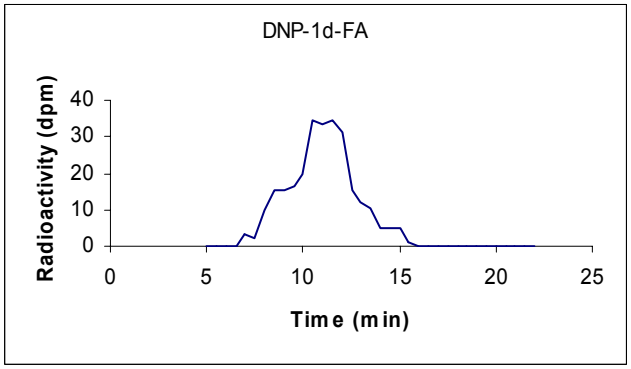
Time (min)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Radioactivity (dpm)
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0
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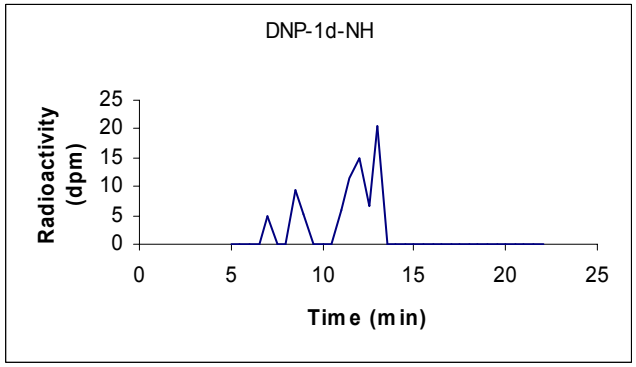
Time (min)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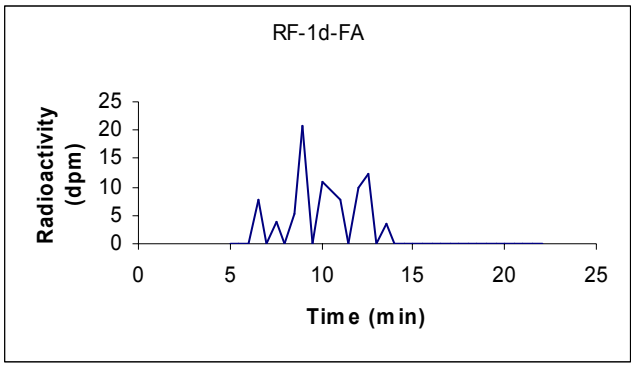
25
Time (min)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Radioactivity (dpm)
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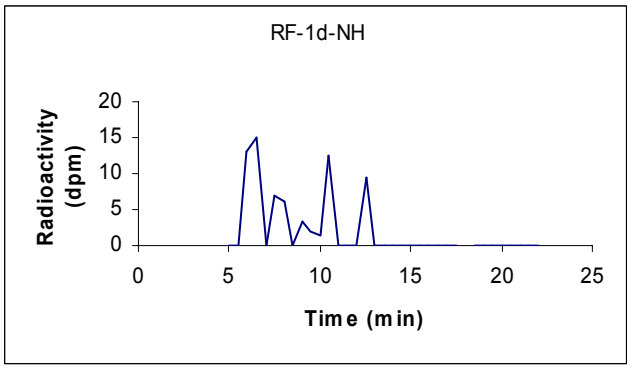
5
10
15
20
25
0
5
10
15
20
25
Time (min)


RF-1d-NH
Radioactivity (dpm)
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15
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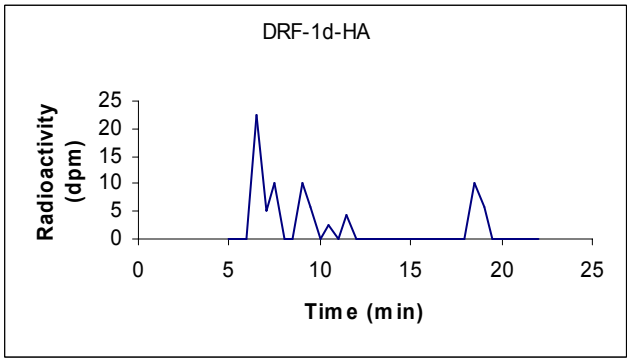
0
5
10
15
20
25
Time (min)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Radioactivity (dpm)
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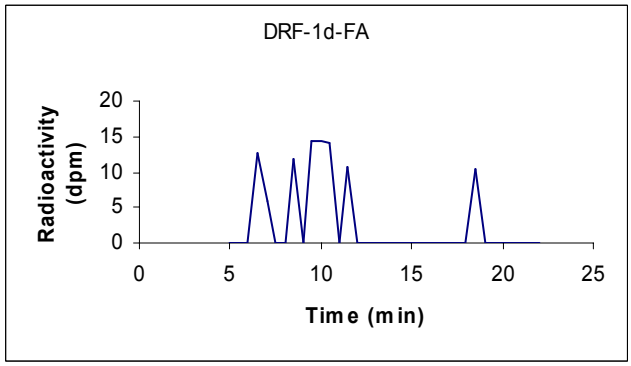
5
10
15
20
25
0
5
10
15
20
25
Time (min)


DRF-1d-FA
Radioactivity (dpm)
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15
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0
5
10
15
20
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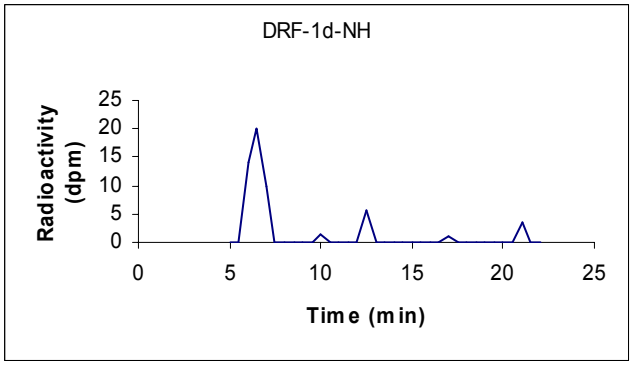
Time (min)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Radioactivity (dpm)
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10
15
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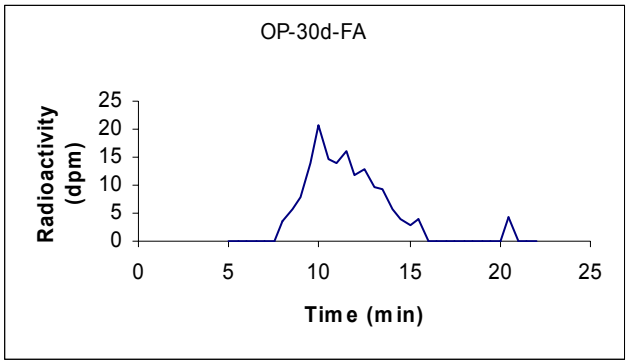
25
0
5
10
15
20
25
Time (min)


OP-30d-FA
Radioactivity (dpm)
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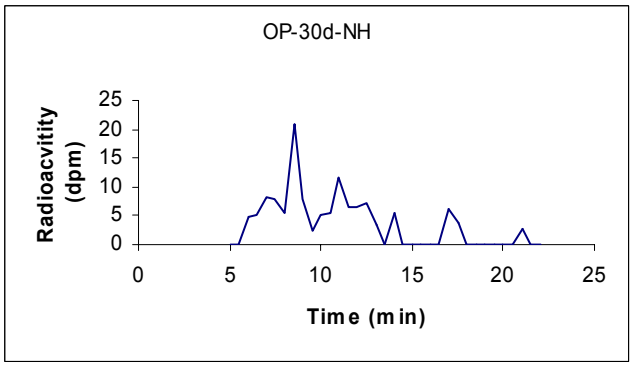
5
10
15
20
25
Time (min)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Radioacvitity (dpm)
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10
15
20
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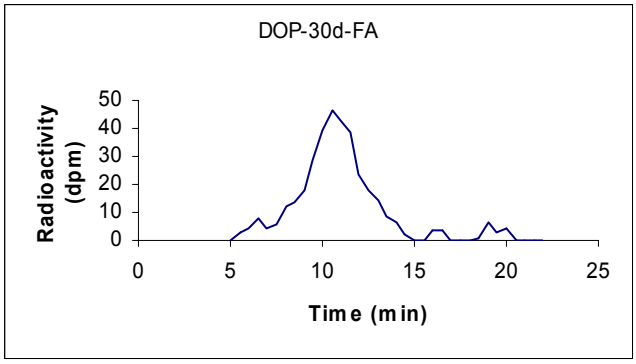
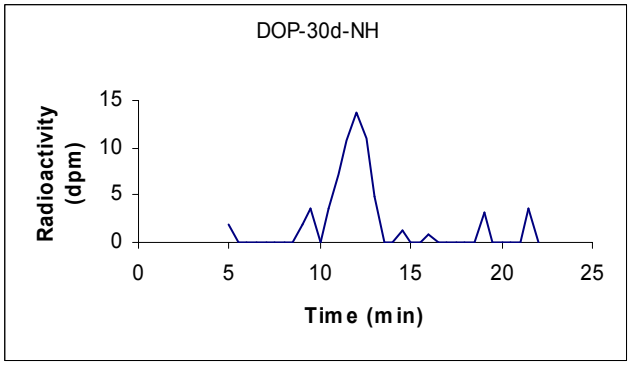
Time (min)


DOP-30d-FA
Radioactivity (dpm)
0
10
20
30
40
50
Time (min)


DOP-30d-NH
Radioactivity (dpm)
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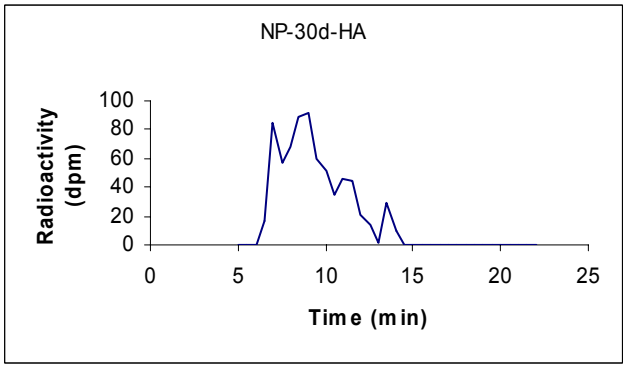
5
10
15
Time (min)


NP-30d-HA
Radioactivity (dpm)
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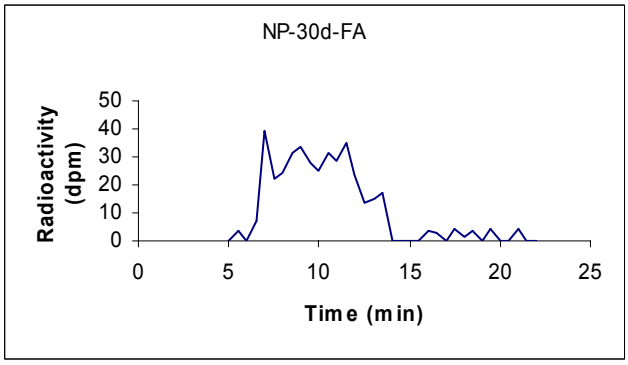
60
80
100
Time (min)


NP-30d-FA
Radioactivity (dpm)
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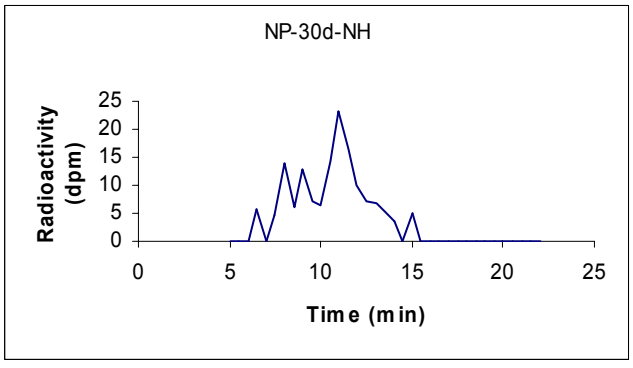
10
20
30
40
50
Time (min)


NP-30d-NH
Radioactivity (dpm)
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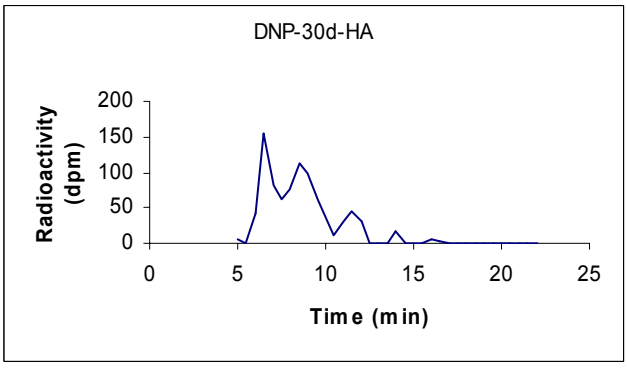
15
20
25
Time (min)


DNP-30d-HA
Radioactivity (dpm)
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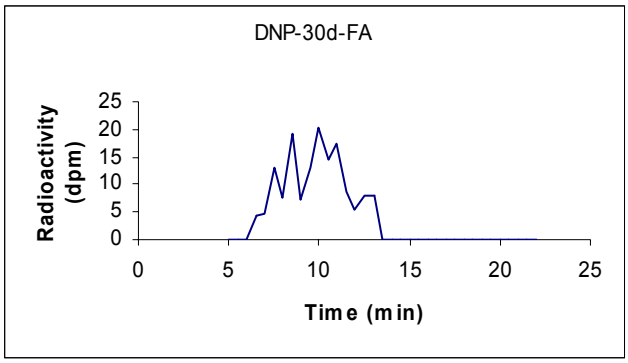
50
100
150
200
Time (min)


DNP-30d-FA
Radioactivity (dpm)
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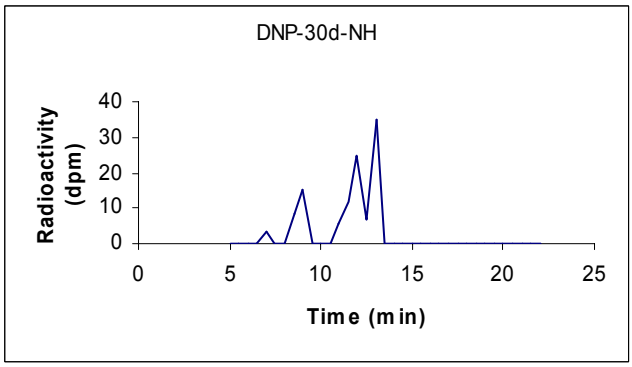
15
20
25
0
5
10
15
20
25
Time (min)


DNP-30d-NH
Radioactivity (dpm)
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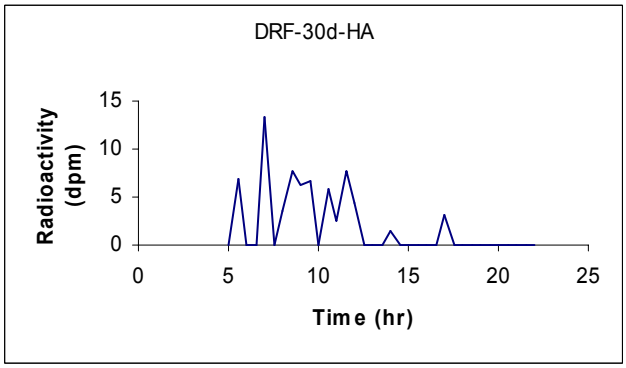
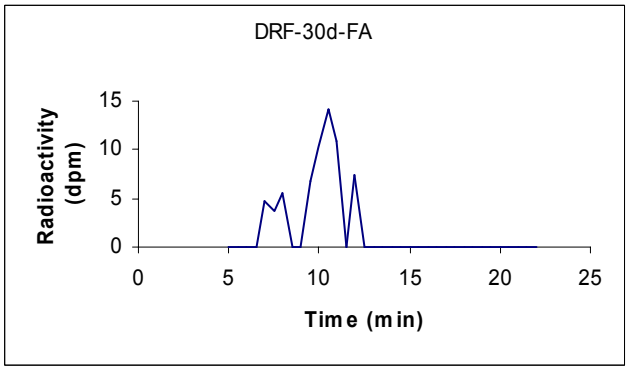
10
20
30
40
0
5
10
15
20
25
Time (min)


DRF-30d-HA
Radioactivity (dpm)
0
5
10
15
0
5
10
15
20
25
Time (hr)


DRF-30d-FA
Radioactivity (dpm)
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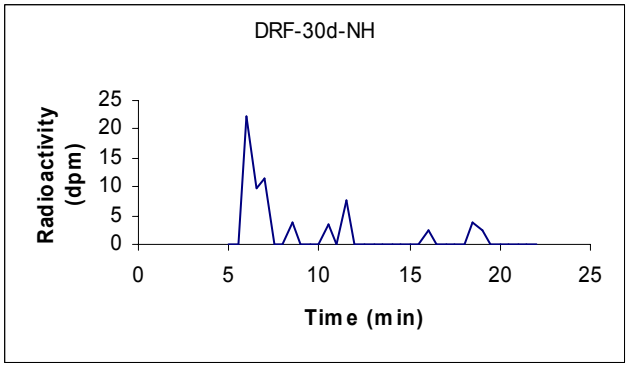
5
10
15
0
5
10
15
20
25
Time (min)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Radioactivity (dpm)
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10
15
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25
0
5
10
15
20
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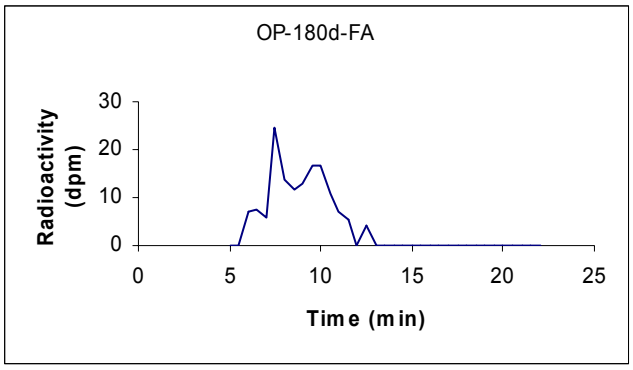
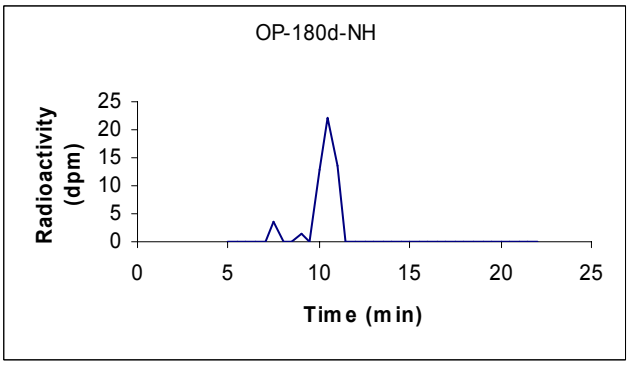
Time (min)


OP-180d-FA
Radioactivity (dpm)
30
20
10
0
0
5
10
15
20
25
Time (min)


OP-180d-NH
Radioactivity (dpm)
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10
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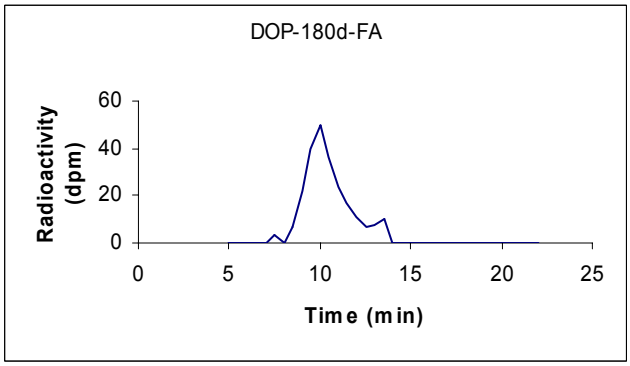
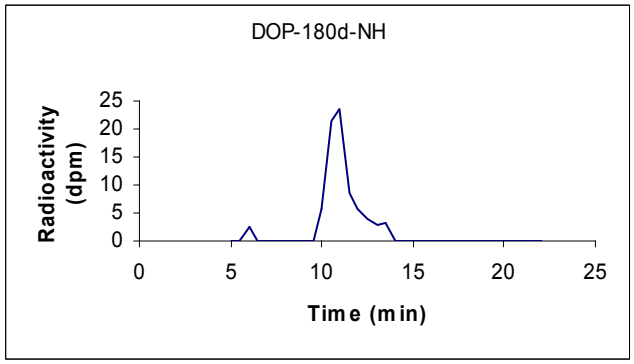
0
0
5
10
15
20
25
Time (min)


DOP-180d-FA
Radioactivity (dpm)
60
40
20
0
0
5
10
15
20
25
Time (min)


DOP-180d-NH
Radioactivity (dpm)
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0
5
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15
20
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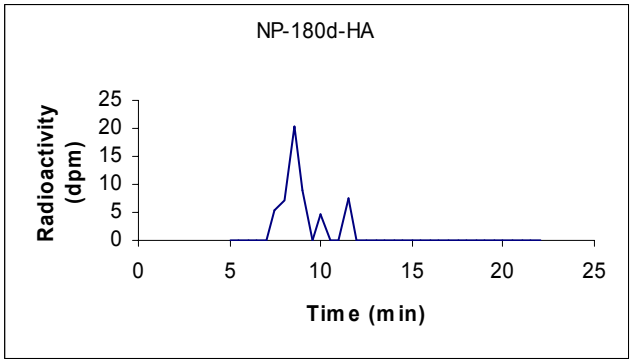
Time (min)


NP-180d-HA
Radioactivity (dpm)
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0
0
5
10
15
20
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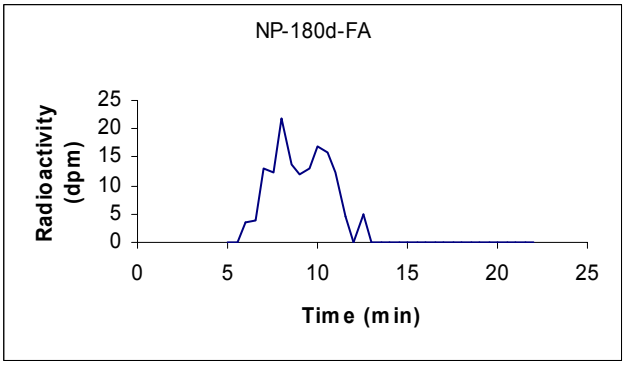
Time (min)


NP-180d-FA
Radioactivity (dpm)
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0
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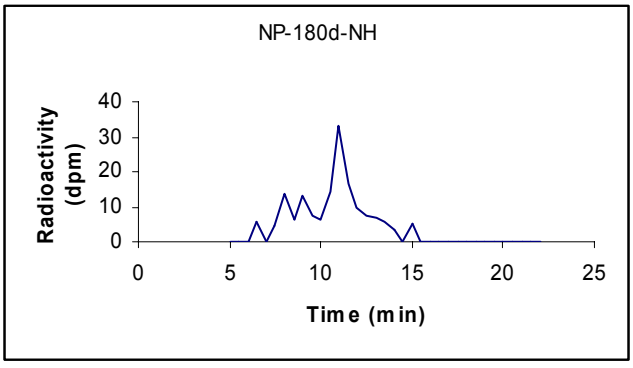
Time (min)


NP-180d-NH
Radioactivity (dpm)
40
30
20
10
0
0
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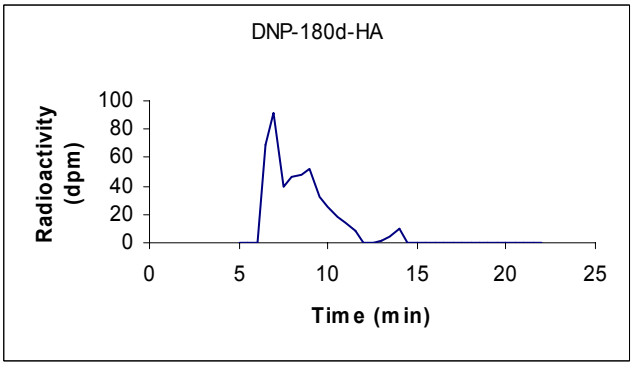
20
25
Time (min)


DNP-180d-HA
Radioactivity (dpm)
100
80
60
40
20
0
0
5
10
15
20
25
Time (min)

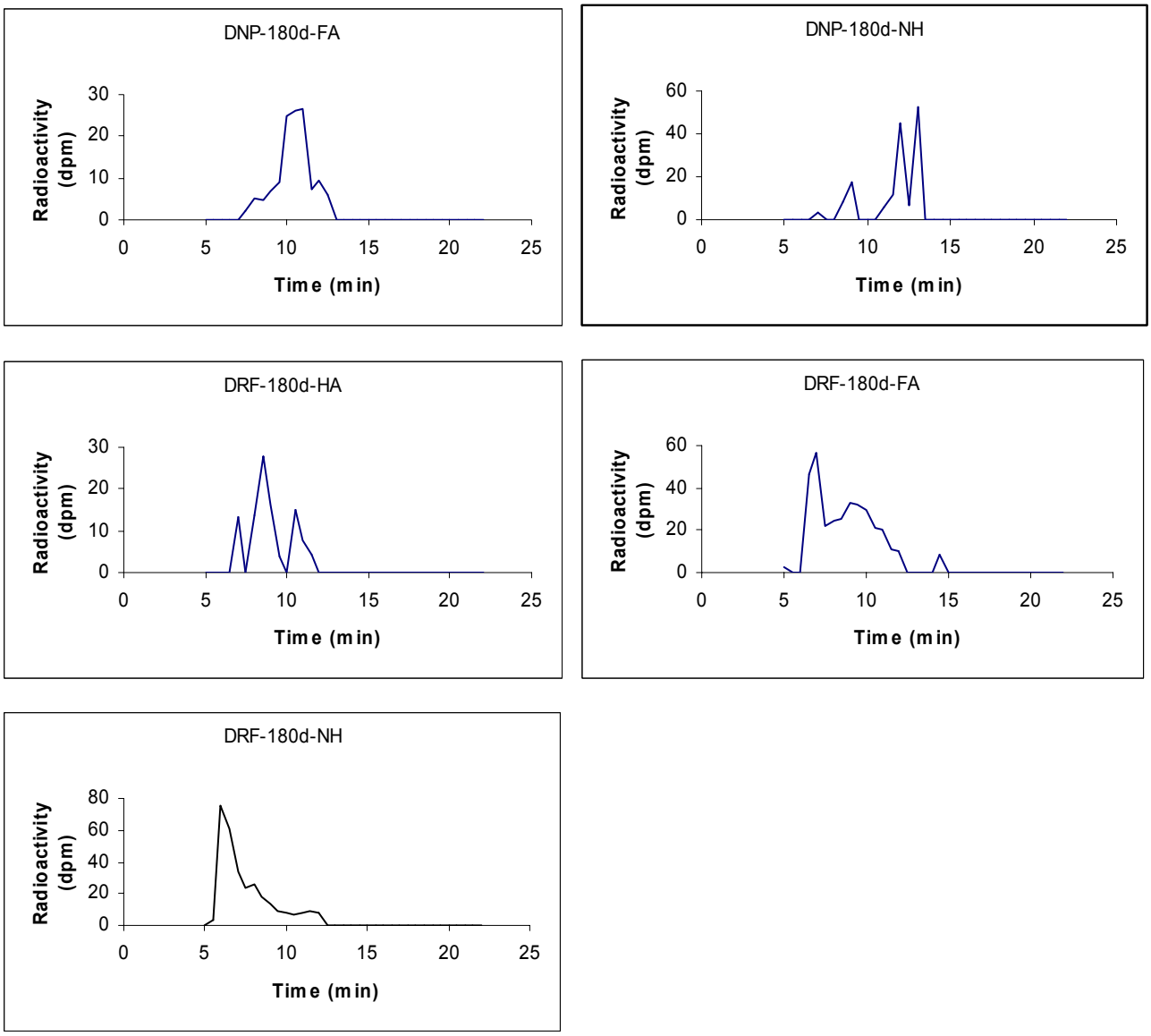


FIGURE S4.3 HPSEC chromatogram of humic substances extracted from MSW Component.

The humic substances were adjusted to pH 6. Eluent was collected and analyzed by scintillation counting. Abbreviations: HA-humic acid; FA-fulvic acid; NH-non-humic substances. OP-fresh office paper; DOP-degraded office paper; NP-fresh newsprint; DNP-degraded newsprint; RF-fresh rabbit food; DRF-degraded rabbit food; 1d-1 day aging; 30d-30 days aging; 180d-180 days aging.

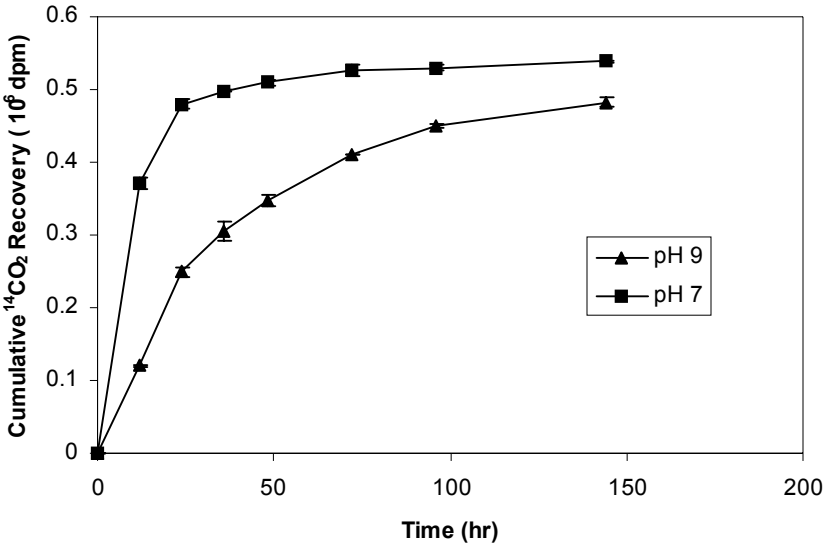


FIGURE S4.4 Comparison of biodegradation of toluene by *PpF1* at different pH.

Data are averages of duplicate samples. Error bars represent standard deviations.

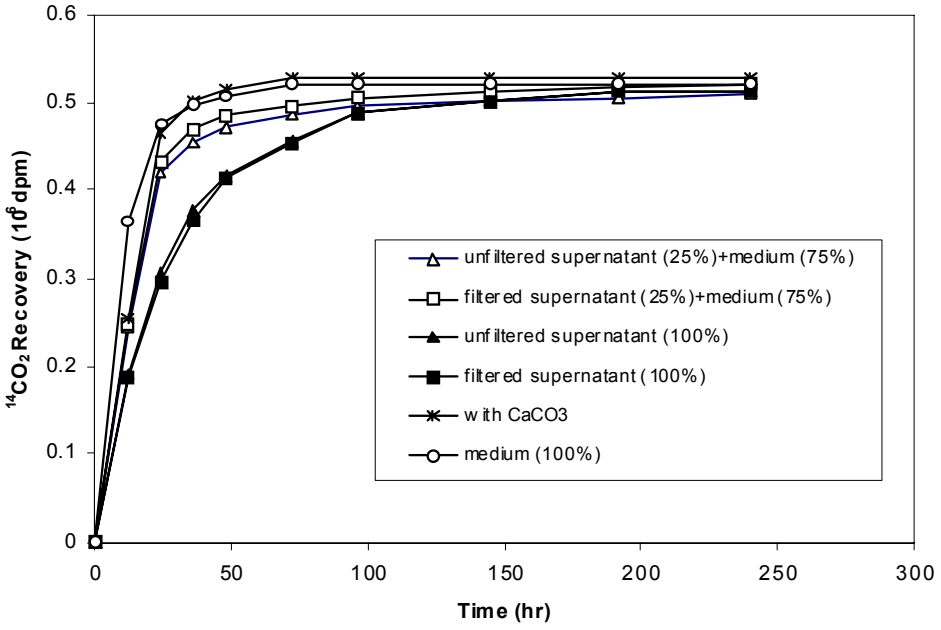


FIGURE S4.5 Comparison of toluene biodegradation in different preparations of fresh office paper blanks.

Office paper and $CaCO_3$ were soaked with growth medium for 30 days. Data are averages of duplicate samples. CV ≤ 22%.

4.4 The Effect of Cellulose Plus Hemicellulose and Lignin on the Bioavailability of Toluene Sorbed to Waste Paper

Abstract

Lignocellulosic materials make up about 38% of municipal solid waste, much of which is disposed in landfills. Organic contaminant sorption to lignocellulosic materials may limit contaminant bioavailability in landfills. The objective of this study was to identify the effect of individual biopolymers in paper on toluene sorption and bioavailability by subjecting office paper and newsprint to enzymatic hydrolysis and acid hydrolysis. Cellulose and hemicellulose exhibited little sorptive capacity for toluene while lignin controlled toluene sorption and bioavailability for both fresh and degraded newsprint. However, the presence of lignin could explain only 54% of the toluene sorptive capacity of degraded office paper, suggesting that crude protein and lipophilic extractives such as sizing agent and/or cell wall compounds were important contributors to the toluene sorption capacity. Lignin extracted from degraded newsprint exhibited a 9.5% lower toluene sorption capacity than that extracted from fresh newsprint, a result that was attributable to incomplete protein removal during lignin purification and the comparatively lower toluene sorption capacity of protein.

Introduction

In the year 2000, there were 1967 operating municipal waste landfills in the US in addition to thousands of closed sites (U.S.EPA, 2002). While modern landfills are designed to contain waste and to collect leachate, this was not always the case. As recently as 1986, many landfills were constructed without an engineered liner (U.S.EPA, 1988). Prior to promulgation of the Resource Conservation and Recovery Act in 1976, the types of waste that could be buried in landfills were not strictly regulated. As a result, groundwater contamination is associated with many older landfills and at least 15% of today's Superfund sites are municipal landfills that received hazardous waste (U.S.EPA 2003).

Some biodegradation and attenuation of organic contaminants occurs in landfills (Hilger and Barlaz, 2001). For example, studies that utilized liquid extracts of refuse microorganisms have documented the anaerobic biodegradation of phenol, toluene, p-cresol (Wang and Barlaz 1998) and selected phthalic acid esters (Ejlertsson *et al*. 1996, Jonsson *et al*. 2003). Landfill leachate also has been shown to harbor microorganisms capable of PCE and TCE dehalogenation (Kromann and Christensen 1998). However, the fact that leachate contains a variety of xenobiotic organic chemicals suggests that biodegradation and other attenuating mechanisms in landfills are incomplete (Hilger and Barlaz, 2001).

One of the primary factors controlling the fate of hydrophobic organic compounds (HOCs) in the environment is sorption to organic matter (Alexander 1995, Luthy *et al*. 1997). Paper is the largest component of municipal solid waste (MSW) (U.S.EPA 2002). As such, the major components of paper, cellulose, hemicellulose and lignin, account for most of the organic matter in landfills. While the decomposition of cellulose and hemicellulose in landfills is well documented (Stinson and Ham 1995, Eleazer *et al*. 1997, Pichler and Kögel-Knabner 2000), their complete biodegradation is not expected as they are present in lignocellulosic complexes. The covalent linkage between polysaccharides and lignin provides physical blockage and renders cellulose and hemicellulose less susceptible to microbial attack (Tong *et al*. 1990, Eleazer *et al*. 1997, Tuomela *et al*. 2000). Concurrently, landfill organic matter decomposition leads to the accumulation of cellular materials such as proteins and lipids (Pichler and Kögel-Knabner 2000).

Recent work has indicated that biopolymers exhibited a wide range of sorptive capacity for HOCs. The partition coefficients describing HOC sorption to cellulose have been reported to be 0.02 mL/g for toluene (Garbarini and Lion 1986); 1.0, 2.6 mL/g for benzene and o-xylene (Xing et al. 1994); 6.45, 3.50, 0.57 mL/g for acetamide pesticides alachlor, metolachlor and propachlor (Torrents *et al.* 1997); 0.25, 0.51, 0.32 for atrazine, prometon and TCE, respectively (Xing et al. 1996). Lignin has much higher sorptive capacity for HOCs relative to cellulose. The partition coefficients were 97.2 mL/g for

toluene (Garbarini and Lion 1986); 402, 460, 140 mL/g for alachlor, metolachlor, and propachlor (Torrents *et al.* 1997); 58.8 and 327 mL/g for benzene and o-xylene respectively (Xing et al. 1994). Wood pulp and fiber is a complex conglomeration of individual biopolymers. The sorption of HOC to wood is consistent with their sorption to individual biopolymers. Mackay and Gschwend (2000) investigated the sorption of monoaromatic hydrocarbons to wood. They found the lignin-water partition coefficient, which was calculated from wood-water partition coefficient and lignin content, agreed with that of the chemically isolated lignin. Severtson and Banerjee (1996) also reported that retention of chlorophenols by wood fibers is governed by the interaction of the acid form of the chlorophenols with lignin.

Despite the large number of landfills that contain organic contaminants, HOC sorption to and desorption from lignocellulosic waste remains largely unexplored. The objective of this study was to identify the effects of individual biopolymers in paper on the sorption and bioavailability of toluene, a model alkylbenzene. Toluene was chosen as the test compound because of its frequent occurrence in landfill leachate (Christensen *et al*. 1994).

Materials and Methods

Experimental Design. Tests were conducted to evaluate the role of cellulose plus hemicellulose and lignin in paper on the bioavailability and sequestration of toluene. Office paper (OP) and newsprint (NP) were selected to represent the range of papers with respect to chemical composition (Table 4.10). OP is a chemical pulp that is nearly completely delignified, while NP is a mechanical pulp that still contains most of the lignin from trees. OP and NP were tested in both fresh and anaerobically degraded forms to evaluate the effect of sorbent decomposition on HOC sorption (degraded office paper and degraded newsprint are referred to as DOP and DNP, respectively).

To evaluate the effect of cellulose and hemicellulose on toluene bioavailability, these compounds were partially removed from paper by enzymatic hydrolysis. First, sorbents containing toluene were generated by exposing each paper type to toluene. Bioavailable

toluene was subsequently consumed by an aerobic toluene degrading bacterium, *Pseudomonas putida* F1 (*PpF1*). Toluene bioavailability was assessed by measurement of the conversion of ^{14}C-toluene to $^{14}CO_2$ (Hatzinger and Alexander 1995). Once daily $^{14}CO_2$ production was less than 0.5% of the initially added toluene, sorbents were subjected to enzymatic hydrolysis to partially degrade the cellulose and hemicellulose. Selective hydrolysis of the organic matrix was intended to investigate whether the removal of cellulose and hemicellulose would enhance the bioavailability of sequestered toluene in paper materials. To evaluate the effect of lignin, toluene bioavailability tests were also conducted with whole NP, DNP, and DOP as well as with the lignins isolated from the three materials. OP was not tested because of its low lignin content (Table 4.10).

Sorbents. NP was collected from The News & Observer Recycling Division (News & Observer Publishing Co., Garner, NC). OP was collected from the NC State University recycling center. The preparation of degraded sorbents and sorbent characterization has been described (Wu *et al*. 2001). All sorbents were dried and ground to pass a 1-mm screen in a Thomas-Wiley laboratory mill and stored in a desiccator until use.

Chemicals and Reagents. All chemicals were reagent grade. ^{14}C-toluene (2.8 mCi/mmol, 99% purity) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). One mCi ^{14}C-toluene was mixed with 10 mL non-labeled toluene (reagent grade) to make a stock solution with a final concentration of 120786 dpm/μL. Stock solutions were stored at -10ºC until use.

Cellulase produced by *Trichoderma reesei* (*T. reesei*) and hemicellulase produced by *Aspergillus niger* (*A. niger*) were purchased from Sigma-Aldrich Chemical Co. An enzyme stock solution was prepared by dissolving 0.045 g of cellulase and 0.09 g of hemicellulase in 15 mL of 50 mM pH 5 citric acid buffer. The cellulase and hemicellulase activities in the stock solution at optimal pH and 37°C were 11.6 and 1.58 units, respectively, where cellulase and hemicellulase units are defined as μmole of glucose produced/(hr· mg cellulase) and μmole of D-galactose produced/(hr· mg hemicellulase), respectively. The optimal pH values for cellulase and hemicellulase are

5.0 and 5.5, respectively. The stock solution was prepared just prior to use to prevent protein denaturation.

Bacterial Strains and Growth Conditions. *PpF1* was grown at 30±1°C in medium containing 4.22 g KH_2PO_4, 3.37 g K_2HPO_4, 0.05 g $CaCl_2 \cdot 2H_2O$, 0.005 g $FeCl_3$, 0.201 g $MgSO_4 \cdot 7H_2O$, 0.5 g NH_4NO_3, 0.01 g disodium EDTA, 1.43 mg H_3BO_3, 0.32 mg $ZnSO_4 \cdot H_2O$, 0.1 mg $CoCl_2 \cdot 4H_2O$, 0.08 mg $CuSO_4 \cdot H_2O$ and 0.05 mg $Na_2MoO_4 \cdot 2H_2O$, per liter of deionized water. To prepare an inoculum for a bioavailability test, 25 mL of sterile medium was inoculated and *PpF1* was grown in an incubator shaker (150 rpm) with 0.12 mmol toluene. After 24 hr, 0.5 mL of the culture was transferred into 25 mL of fresh medium and incubated for an additional 24 hr. The cells were then washed twice with 50 mM pH 7 phosphate buffer and resuspended to an optical density of 0.79 at 600 nm.

Optimization of Enzymatic Sorbent Hydrolysis. *PpF1* grows optimally at pH 7 and 30°C in the presence of oxygen, which was provided by constant shaking. As described above, the pH optima for cellulase and hemicellulase are pH 5 and 5.5, respectively. Thus, it was necessary to evaluate whether the enzymes could be used under conditions appropriate for *PpF1*. In this study, the effect of pH was tested because it is difficult to adjust in the presence of strong phosphate buffer. The temperature was adjusted from the optimal temperature for *PpF1* to 37°C for enzymatic hydrolysis.

To evaluate the effect of pH, the enzymatic hydrolysis was carried out in 160 mL glass ampoules, in which suspensions were continuously mixed with a magnetic stir bar (Kaya *et al*. 1996). Three gm of sorbent that had been sterilized by 2.2 Mrad of γ–irradiation from a ^{60}Co source were mixed with 60 mL of 50 mM pH 5 citric acid buffer or pH 7 phosphate buffer depending on the target pH. One mL of 3% sodium azide was added to prevent aerobic degradation of the sorbent and hydrolysis products. Three mL cellulase plus hemicellulase filter-sterile stock solution (0.2 μm HT Tuffryn membrane syringe filter, Ann Arbor, MI) was incubated with the sorbent/buffer mixture at 37°C at either pH 5 or 7 for about 100 h. Solid samples without enzyme addition served as controls.

Samples (1.5 mL) were removed periodically to measure the release of reducing sugars.

Preparation of Sorbents with Sequestered Toluene. Sorbents containing sequestered ^{14}C-toluene were prepared by depleting the readily bioavailable toluene under conditions designed to promote toluene biodegradation. After toluene had been aged with sorbents for 1, 30 and 180 days, four replicate samples were inoculated with 3 mL of a suspension of *PpF1* to give 1.5×10^8 cells/mL. Bottles were sealed with a stainless steel Swagelok™ cap into which three stainless steel needles were welded (Fig. 4.4). Sterile glass vials were attached to the two long needles. One vial contained 2 mL of 2 N NaOH to dissolve the evolved $^{14}CO_2$. The second vial contained 1mL of 30% (v/v) H_2O_2 plus 0.5 mL of 0.5% (wt/wt) $FeCl_2$ that acted as a catalyst to promote oxygen production from H_2O_2 to maintain aerobic conditions. The long needles were sealed with push button syringe valves to prevent volatilization losses. A Tedlar gasbag was attached to the short needle to prevent a pressure increase in the reactor system from excess O_2. Samples were incubated at 30±1°C in an incubator shaker (New Brunswick Scientific C24 Classic series, Edison, NJ) operated at 140 rpm. ^{14}C-toluene biodegradation was quantified by measurement of $^{14}CO_2$ dissolved in the NaOH trap, the contents of which were removed at 0.2 – 2 day intervals. Duplicate 0.5 mL NaOH aliquots were mixed with 6 mL of Ultima Gold scintillation cocktail (Packard BioScience, Meriden, CT). Prior to scintillation counting, samples were refrigerated overnight to reduce chemiluminescence. Fresh NaOH was added after each sampling.

Enzyme Facilitated Bioavailability Test. To evaluate whether cellulose and hemicellulose hydrolysis would increase toluene bioavailability, hydrolytic enzymes were added to the sorbent containing toluene that remained once toluene degradation became negligible in bioavailability tests. After consumption of bioavailable toluene as described above, the pH of the sorbent/growth medium mixture was adjusted to 5 with 6 N HCl to provide suitable conditions for enzymatic hydrolysis. Three mL of enzyme stock solution were then added to two of the four replicate bioavailability reactors while the remaining two served as enzyme-free controls. An autoclaved Teflon™ -coated stirrer was added to the reactors aseptically. All samples were then incubated at 37°C while

being stirred continuously. After 48 hr of enzyme treatment, the pH of the sorbent/medium mixture was adjusted back to pH 7 with 6 N NaOH. To evaluate the effect of enzymatic hydrolysis, samples were reinoculated with 3 mL of *PpF1* and toluene mineralization was again monitored by measuring $^{14}CO_2$ production.

Lignin Isolation. Lignins were isolated from NP, DNP and DOP by removing cellulose and hemicellulose with sulfuric acid (Petterson 1984). Ground sorbent (1g) was extracted with 150 mL of a 2:1 mixture of toluene/95% ethanol for 20 min to remove lipophilic substances. The solvent extracted sorbents were then hydrolyzed with 10 mL of 72% (w/w) H_2SO_4. The hydrolyzed mixture was diluted with 300 mL deionized water and autoclaved for 60 min at 121°C and 103.4 Kpa. The mixture was filtered through a glass fiber filter (Whatman 934AH) and the collected lignin was rinsed continuously with deionized water. The lignin (NP-lignin, DNP-lignin, DOP-lignin) was then dried at 75°C and stored in a desiccator until use.

Reducing sugar measurement. Reducing sugars released during hydrolysis were measured by the dinitrosalicyclic acid (DNS) assay (Chaplin and Kennedy 1994). Samples were prepared by centrifugation at 16000 ×g for 5 min. To measure reducing sugars, 1.0 mL of the DNS reagent was mixed with 100 μL sample and heated at 100°C for 10 min. After rapid cooling to room temperature, the absorbance was measured at 570 nm. A standard curve was prepared with α-D-glucose.

Data Analysis. The extent of sorbed toluene mineralization was calculated from eqn. 1.

$$Extent\ of\ mineralization\ (\%) = \frac{P_{sorbent} - P_{blank}}{q} \times 100 \qquad (1)$$

Where $P_{sorbent}$ and P_{blank} represent $^{14}CO_2$ (dpm) production from samples containing sorbents and sorbent-free blanks that had the same aqueous-phase toluene concentration as the sorbent-containing samples after aging, and q (dpm) corresponds to the amount of toluene present in a sorbent after aging. Thus, the extent of mineralization at the end of the bioavailability test was calculated based on the conversion of sorbed toluene to $^{14}CO_2$.

Differences between treatments were evaluated by performing analyses of variance (ANOVA) ($p < 0.05$). All statistical tests were performed by SAS 8.0 software (SAS Institute Inc., Cary, NC).

Results and Discussion

Optimization of Cellulose Hydrolysis. Initially, tests were conducted to identify suitable conditions for enzymatic hydrolysis. These tests were necessary to understand whether it would be possible to maintain one set of environmental conditions in a reactor that would allow for both biological activity of *PpF1* and enzyme activity. Enzyme activities were tested at pH 5 and 7, which are the pH optima for cellulase activity (Geimba *et al*. 1999) and *PpF1*, respectively. The pH optimum for hemicellulases (5.5) was slightly compromised because the sorbents contained less hemicellulose than cellulose. Sugar release was significantly higher at pH 5 for all sorbents (Table 4.10).

Enzymatic sugar release exhibited an initial rapid phase and then declined as time elapsed (Fig 4.4). Possible explanations for the decline in enzyme activity include end product inhibition (Azevedo *et al*. 2002), substrate transformation (Puri 1984) and reduced substrate surface area. The major products of cellulose hydrolysis, cellubiose and glucose, can bind to endoglucanases and cellobiohydrolases and inhibit cellulase adsorption to the sorbent (Sharrock, 1988; Azevedo *et al*. 2002). Furthermore, cellubiose is reported to be a stronger inhibitor than glucose (Desai and Converse 1997). The cellulase used in this study, originating from *T. reesei*, is relatively deficient in β-glucosidase, a major component in cellulase that is responsible for the conversion of cellubiose to glucose (Ooshima *et al*. 1991). Thus, end product inhibition is one likely explanation for the decline in enzyme activity. Replacing the hydrolysate with end product-free buffer may have enhanced the extent of cellulose and hemicellulose hydrolysis in each sorbent by eliminating end product inhibition. However, hydrolysate replacement would have been impractical for assessing the effect of enzymatic hydrolysis on toluene bioavailability because any toluene that would have been released to the liquid phase would have been removed. Substrate transformation during hydrolysis would

render sorbents also less susceptible to enzyme attack. The preferential degradation of the more reactive amorphous cellulose region leaves the sorbents enriched in crystalline cellulose, which is more resistant to further hydrolysis (Nazhad *et al*. 1995). Finally, enzymes must bind to the sorbent surface to act on cellulose, and the surface area available for enzyme sorption decreases during hydrolysis (Sinitsyn *et al*. 1989). The enzyme system for the degradation of hemicellulose is a broad group of synergistic enzymes (Puls and Poutanen 1989). Like most other polysaccharide-degrading enzymes, hemicellulase is also inhibited by the buildup of reaction products (Sreenath *et al*. 1999).

The data in Table 4.10 show a substantial decrease in enzyme activity when the pH was increased from 5 to 7. Thus, it was necessary to adjust conditions for enzymatic hydrolysis in the sorbent/medium mixture to pH 5. The extent of hydrolysis measured at pH 5 and 37°C was judged to be sufficient to study the effect of cellulose and hemicellulose hydrolysis on toluene release.

Enzyme-Facilitated Bioavailability Tests. The effect of enzyme addition on the bioavailability of toluene sorbed to NP after aging times of 1, 30 and 180 days is presented in Fig 4.5. (The effect of aging time on bioavailability was investigated by Chen (2003) and bioavailability was found to decrease with increasing aging time). In no case was there a statistically significant effect that could be attributed to the addition of cellullase and hemicellulase. Similar results for OP, DOP, and DNP are presented in Figs S4.6, S4.7, S4.8 (Supporting Information).

The presence of reducing sugars in bioavailability tests was further confirmed by the recovery of non-labeled CO_2 in the base trap as measured by total inorganic carbon after reinoculation of *PpF1*. Preliminary tests showed that *PpF1* does not have the ability to utilize cellulose and hemicellulose. Therefore, the non-labeled CO_2 must have originated from the metabolism of reducing sugars released during hydrolysis. Although sorbent organic matter was effectively destroyed, $^{14}CO_2$ production from ^{14}C-toluene was not enhanced. To confirm that ^{14}C-toluene biodegradation was controlled by substrate availability and not by the loss of toluene degrading activity after preferential metabolism of easily degradable sugars, samples from bioavailability tests, collected after enzymatic

hydrolysis, were analyzed by gas chromatography to determine the toluene concentration. The absence of detectable toluene (less than 4 μg/L) confirmed that no toluene was released during sorbent hydrolysis (the calculated equilibrium aqueous toluene concentration should be 16521- 54250 μg/L based on the published isotherm data for each sorbent (Wu *et al*. 2001)). This suggests that cellulose and hemicellulose do not control the sequestration of toluene for the tested sorbents.

Bioavailability of Toluene Sorbed to Purified Lignin. To evaluate the significance of lignin as the component of paper that controls sorption, bioavailability tests were conducted with NP, DNP, and DOP as well as with their extracted lignins. The mass of purified lignin used was equivalent to the mass of lignin in sorbents as calculated from reported lignin contents (Table 4.10). Both sorbent and lignin were spiked with the same amount of toluene prior to aging.

The fraction of the added toluene that was sorbed to each material after 1 day of aging is presented in Table 4.11. The difference in the fraction of toluene sorbed to paper materials and their respective lignins was statistically insignificant for NP ($p = 0.92$) and DNP ($p = 0.84$), indicating that extracted lignin could account for all of the sorption capacity in NP and DNP. Similar results were also observed after an aging time of 30 days with NP and NP-lignin (Table S4.1 in Supporting Information). The results of toluene bioavailability tests with NP, DNP, and their isolated lignins after 1 day aging are presented in Fig 4.6. Mineralization data are corrected for sorbent-free blanks so that the $^{14}CO_2$ production is based on the conversion of sorbed toluene. As illustrated in Fig 4.6, no statistically significant difference was observed for toluene bioavailability between NP and NP-lignin ($p = 0.99$) or DNP and DNP-lignin ($p = 0.92$). Similar results were also obtained for NP and NP-lignin after an aging time of 30 days (Fig S4.9 in Supporting Information).

Both the sorption capacity and bioavailability data suggest that lignin controls toluene sorption to and desorption from NP and DNP. In the mechanical pulping process used to produce NP, wood is fragmented and the treated fiber is released with little removal of

lignin. Therefore, all major wood components, e.g. cellulose, hemicellose, lignin, are present in NP. In contrast, other organic matter fractions that can sorb toluene, such as crude protein and lipophilic extractives were present in relatively small amounts in NP (Table 4.10). The HOC sorption capacity of cellulose plus hemicellulose and lignin are explained by their structural differences. Although cellulose is completely insoluble in water, its molecules have a high affinity for water (Kuhad and Singh 1993). The polyhydroxyl structure together with other polar groups specifically interacts with water molecules via H-bonding. The strong interaction between the cellulose surface and water limits the interaction of cellulose with non-polar toluene molecules, leading to the relatively low sorptive capacity of cellulose. Hemicellulose has a similar molecular structure to cellulose although hemicellulose is composed of variable sugar subunits instead of repeating glucose subunits. Consequently, hemicellulose also exhibits little HOC sorption capacity (Mackay and Gschwend 2000). In contrast, the phenylpropane units of lignin produce a relatively hydrophobic region that has a higher affinity for HOCs than the hydrophilic structure of cellulose (Grathwohl 1990). The greater toluene sorption capacity of lignin can also be explained on the basis of solubility parameters, where sorbate/sorbent systems become more compatible as the difference between sorbate and sorbent solubility parameter decreases. The solubility parameters for toluene, cellulose and lignin are 8.8, 14.5-16.5 and 10-12 $(cal/cm^3)^{1/2}$ respectively (Barton 1983). This is consistent with the higher toluene sorption capacity of lignin relative to cellulose because the solubility parameter difference between toluene and cellulose/hemicellulose is greater than that between toluene and lignin.

The partition coefficients (K_p) describing toluene sorption to lignin are different among sorbents (Table 4.11). Such variation is consistent with previous reports, in which K_p values of 97.2, 141 and 84.2 mL/g have been reported for toluene sorption to Kraft pine lignin, organosolv lignin and alkali lignin, respectively (Garbarini and Lion 1986, Xing *et al*. 1994). This variation is not surprising as the composition of lignin varies depending on plant source, plant age, and isolation technique (Dence 1992). The ideal isolation method would allow the collection of chemically unmodified lignin that is free from contaminants. However, none of the existing methods fulfill this requirement (Tuomela *et*

al. 2000). E.g.; the acid hydrolysis lignin extraction procedure used here does not completely remove cellulose/hemicellulose and proteins (Dence 1992). It should also be noted that this procedure modifies lignin present in plant species because ether linkages are cleaved and condensation reactions result (Wu and Argyropoulos 2003). For paper products, however, this structural modification may be less significant because of the prehydrolysis process employed prior to mechanical treatment of wood in the production of coarse fibers (Wallis 1971). Prehydrolysis involves treatment of wood under high pressure and temperature. Lignin solvolytic reactions take place by the action of water at elevated temperature. This treatment liberates components from the wood that render the solution mildly acidic and catalyze condensation reactions (Wallis 1971). After prehydrolysis, lignin becomes more stable under acidic conditions as α and β-ether linkages that are sensitive to acid condensed to form carbon-carbon bonds. As a result, the extracted NP- and DNP-lignins may have adequately represented the lignin originally present in NP and DNP.

There are two potential explanations for the lower sorptive capacity of DNP-lignin relative to NP-lignin. First, although lignin biodegradation is extremely slow under anaerobic conditions, some transformation and even mineralization has been reported (Benner *et al* 1984, Akin and Rigsby 1987, Akin and Benner 1988, Butler and Buckerfield, 1979, Dittmar and Lara 2001). The cleavage of aromatic rings in lignin could result in a lower toluene sorption capacity. However, analysis of NP-lignin and DNP-lignin by NMR showed no structural differences (data not shown), indicating lignin was either highly resistant to microbial attack or the biodegradation rate was extremely slow. Second, elemental analysis showed that DNP-lignin has a higher nitrogen content (0.63%) relative to NP-lignin (0.19%), and the C/N ratio of NP-lignin decreased from 318 to 96 during biodegradation. The accumulation of nitrogen is likely a combination of microbial protein resynthesis during biodegradation and the incomplete removal of protein during the acid hydrolysis procedure (Dence 1992, Martens 2002). Difference in toluene sorption capacity between protein and lignin may therefore explain the decreased sorptive capacity of DNP-lignin relative to NP-lignin.

In the case of DOP, the DOP lignin accounted for only 54% of the total DOP sorption capacity (Table 4.11). There are several explanations for this. First, hydrophobic sizing agents are applied to cellulose fibers during OP production to prevent water penetration. These agents include alkenyl succinic anhydrides (ASA) and alkyl ketene dimmers (AKD), all of which can be expected to contribute to the sorptive capacity of DOP because of their hydrophobicity. Second, the ratio of lipophilic extractives plus protein to lignin in DOP was much larger than in NP and DNP, suggesting that both lipophilic extractives (sizing agents, cell wall constituents) and protein contributed to the HOC sorption capacity of DOP. Similarly, because of the presence of crude protein and lipophilic extractives, bioavailability tests conducted with DOP composite showed a lower initial mineralization rate of sorbed toluene relative to DOP-lignin (Fig 4.6, $p < 0.01$). Also at the termination of the bioavailability test, the extent of sorbed toluene mineralization was lower for DOP compared to DOP-lignin. The contribution of crude protein to the decrease of toluene bioavailability in DOP is likely not significant because amorphous protein is plasticized by water at room temperature (Green *et al*. 1994, Sochava, 1997) and desorption of HOCs from the comparatively expanded, flexible organic matter in a rubbery polymer is fast (Xing and Pignatello 1997). In contrast, lipophilic extractives provide a hydrophobic environment in which nonionic organic compounds may strongly sorb. Sorbed toluene in DOP may have been less bioavailable than in DOP-lignin because of the strong hydrophobic interactions between lipophilic extractives and toluene. It has been reported that intermolecular interactions may be strong enough to resist exhaustive solvent extraction (Guthrie *et al*. 1999).

Environmental Significance. In addition to lignocellulosic materials, landfills contain plastics that will have considerably higher sorption capacities (Wu *et al*. 2001). Interestingly, older landfills that are most likely to contain HOCs and were likely built without engineered liners, will have lower concentrations of plastics due to their relatively recent development as a packaging material. Reinhart *et al*. (1990) reported that increases in sorbent hydrophobicity as a result of biopolymer degradation had little effect on K_{oc} for sorption of HOCs to relatively nonpolar refuse, suggesting that landfill leachate HOC concentrations could increase over time due to the loss of sorbent organic

matter. For a landfill where paper waste dominates, however, the biodegradation of cellulose and hemicellulose should not affect the overall sorptive capacity given the persistence of lignin and the presence of resynthesized lipids and proteins that have higher affinity for HOCs. Thus, where paper waste is dominant, HOC concentrations in leachate can be expected to decrease with time.

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4.5 Figures and Tables of Manuscript 2

FIGURE 4.4 Bioavailability Reactor.

Same as FIGURE 3.1.

TABLE 4.10 Sorbent Composition and Effect of pH on Enzymatic Hydrolysis of Biopolymers

sorbents	sorbent characterization (%) [a]					pH 5 [b]	pH 7 [b]
	cellulose	hemicellulose	lignin	lipophilic extractives	crude protein		
office paper	64.7	13.0	0.93	0.7	0.31	19.1 (0.2)	7.6 (0.1)
degraded office paper	36.2	6.9	4.8	3.3	4.99	14.7 (0.5)	7.0 (0.1)
newsprint	48.3	18.1	22.1	1.6	0.44	22.0 (0.1)	7.7 (0.1)
degraded newsprint	35.1	16.0	32.3	1.4	3.74	8.5 (0.8)	4.0 (0.3)

[a] From Wu *et al*. (2001). [b] Data are the percentage of cellulose plus hemicellulose in sorbents converted to reducing sugars. Data are the averages of triplicate samples and values in parenthesis are the standard deviations.

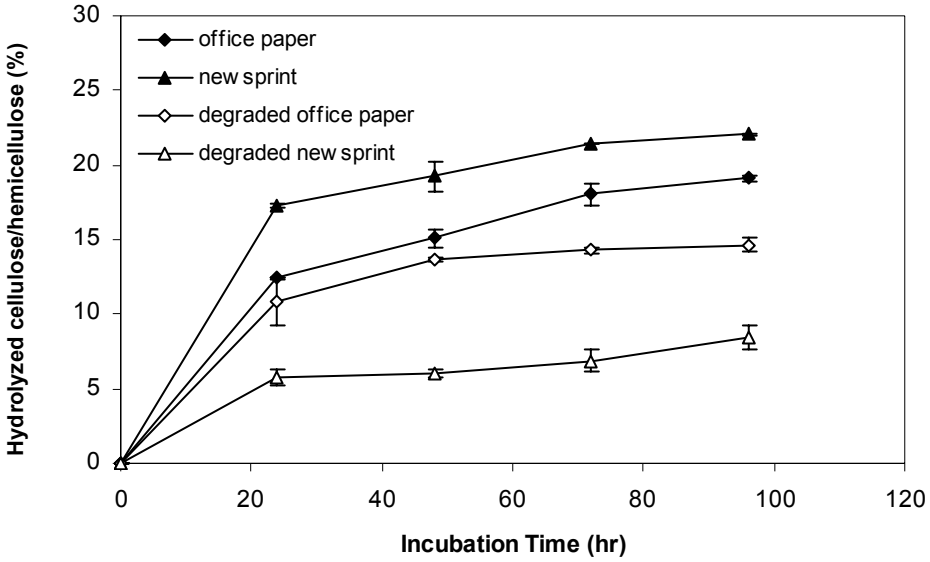


FIGURE 4.4 Comparison of enzymatic sorbent hydrolysis at pH 5 and 37°C

Data are averages of triplicate samples. Error bars correspond to standard deviation.

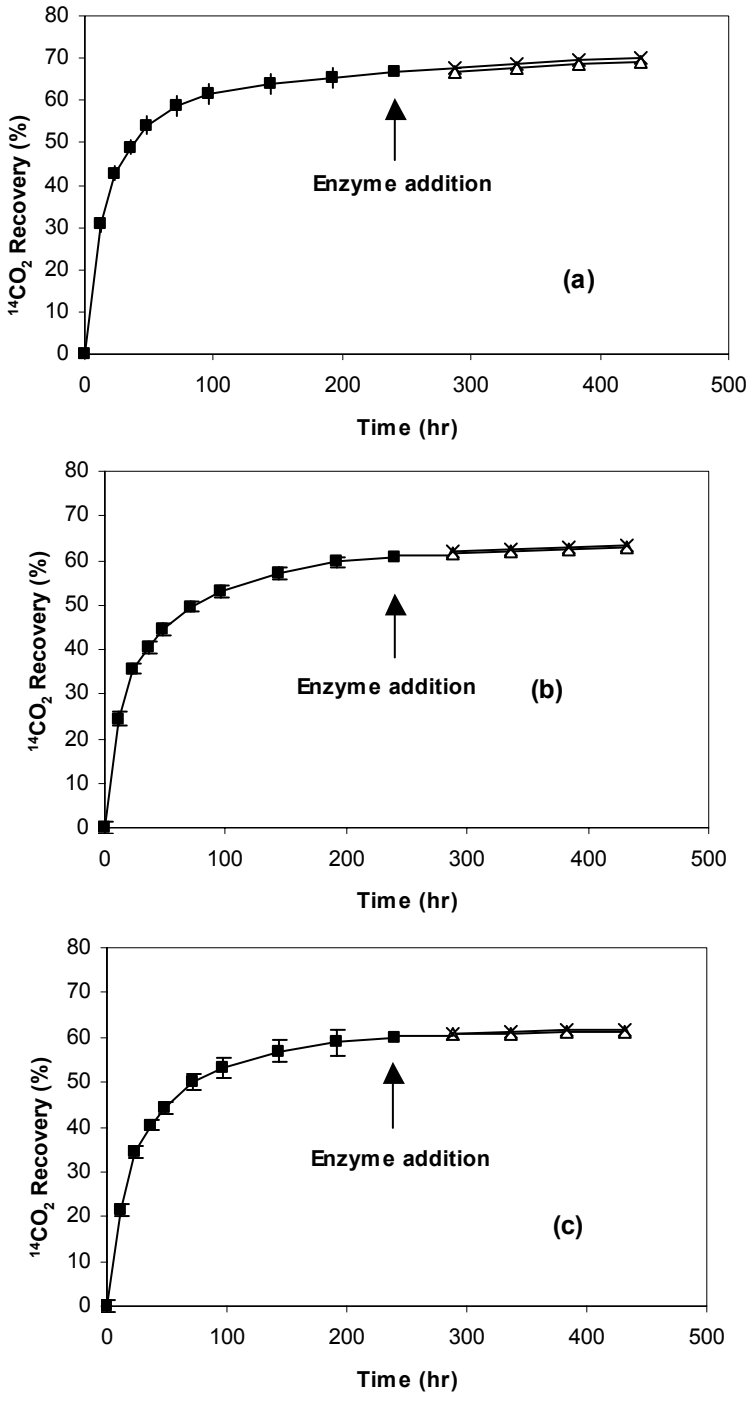


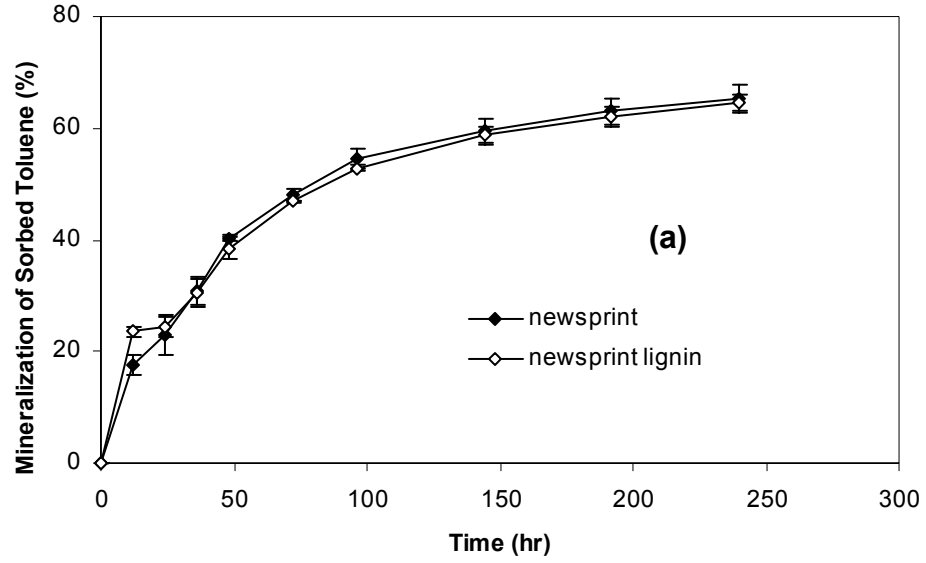
FIGURE 4.5 Effect of cellulase and hemicellulase addition on the bioavailability of toluene sorbed to newsprint aged for (a) 1 day, (b) 30 days and (c) 180 days.

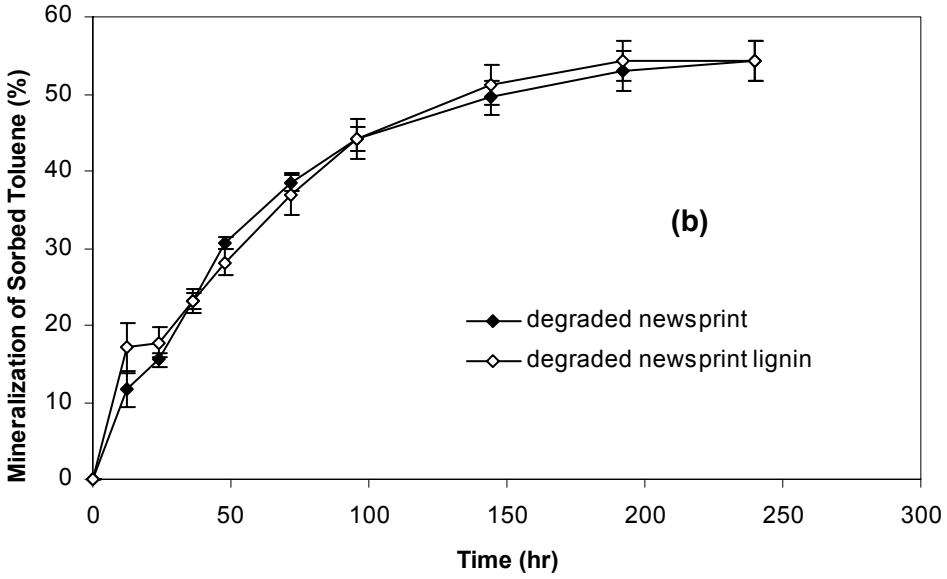
Data are presented as the percentage of toluene added initially recovered as $^{14}CO_2$. Data before enzyme addition (■) are averages of four replicates. Data with enzyme addition (Δ) and data without enzyme addition (×) are averages of duplicate samples. Error bars correspond to standard deviations. Some error bars are too small to view.

TABLE 4.11 Sorption of Toluene to MSW Components and Their Lignins (%) [a, b]

sorbents	toluene uptake (%)		K_p (mL/g) [d]
	whole sorbent [c]	lignin extracted from sorbent	
newsprint	41.99 (0.33)	40.84 (0.13)	82.84 (1.05)
degraded newsprint	47.43 (0.65)	47.40 (0.50)	75.01 (1.08)
degraded office paper	37.81 (0.24)	20.62 (1.53)	143.95 (5.23)

[a] Data are the percentage of sorbed toluene based on total toluene added. [b] Data are the averages of duplicate samples and values in parenthesis are the standard deviations. [c] Mass of sorbents is similar to those used in bioavailability tests (3 g). [d] K_p values were calculated for the sorption of toluene to extracted lignin.


Mineralization of Sorbed Toluene (%)
80
60
40
20
0
0
50
100
150
200
250
300
Time (hr)
(a)
newsprint
newsprint lignin


Mineralization of Sorbed Toluene (%)
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (hr)
(b)
degraded newsprint
degraded newsprint lignin

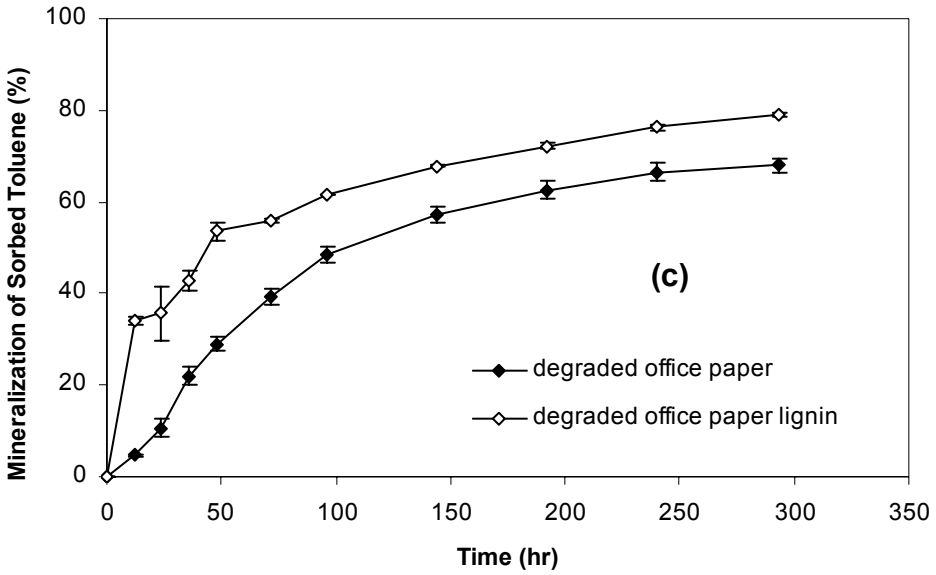


FIGURE 4.6 Bioavailability of toluene sorbed to (a) fresh newsprint, (b) degraded newsprint and (c) degraded office paper and isolated lignins of equivalent mass.

Mineralization data were calculated based on equation 1 and data are averages of three replicates. Error bars correspond to standard deviation. Some error bars are too small to view.

4.6 Supporting Information for Manuscript 2

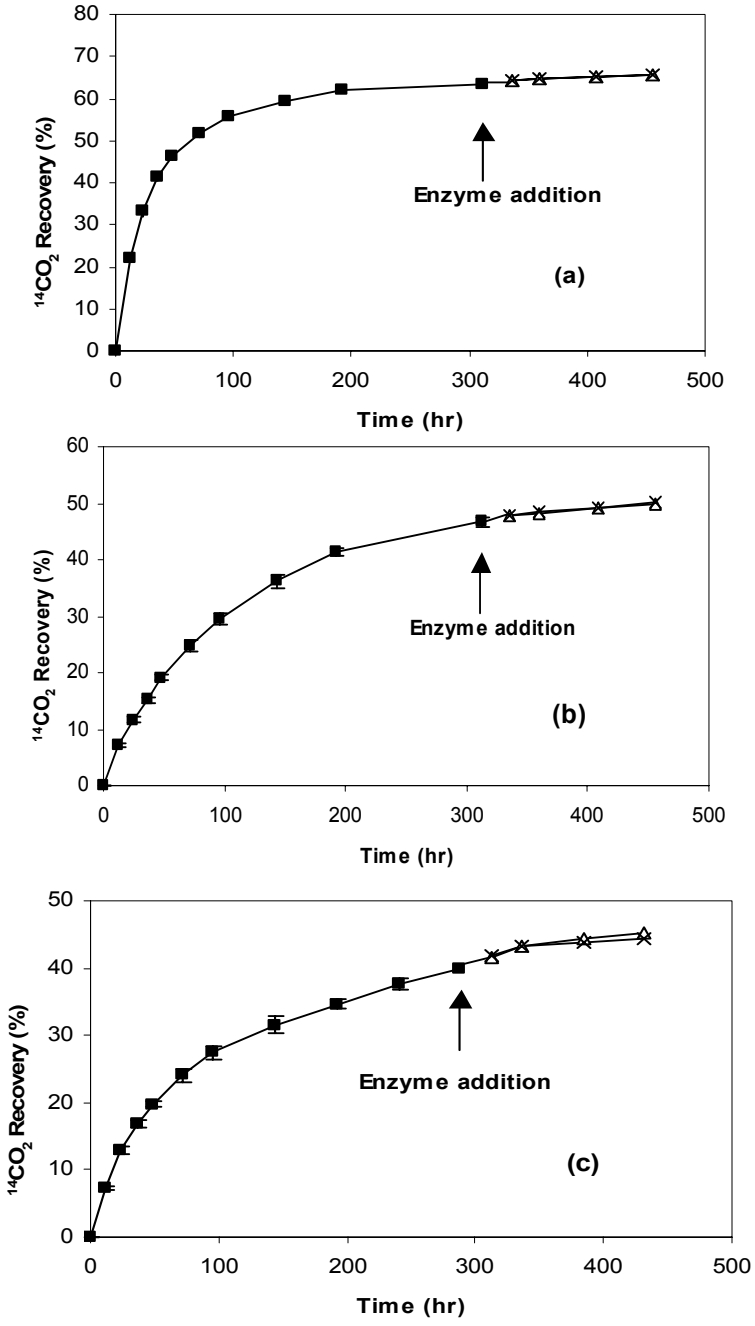


FIGURE S4.6 Effect of cellulase and hemicellulase addition on the bioavailability of toluene sorbed to office paper aged for (a) 1 day, (b) 30 days and (c) 180 days.

Data are presented as the percentage of toluene added initially recovered as $^{14}CO_2$. Data before enzyme addition (■) are averages of four replicates. Data with enzyme addition (Δ) and data without enzyme addition (×) are averages of duplicate samples. Error bars correspond to standard deviations. Some error bars are too small to view.

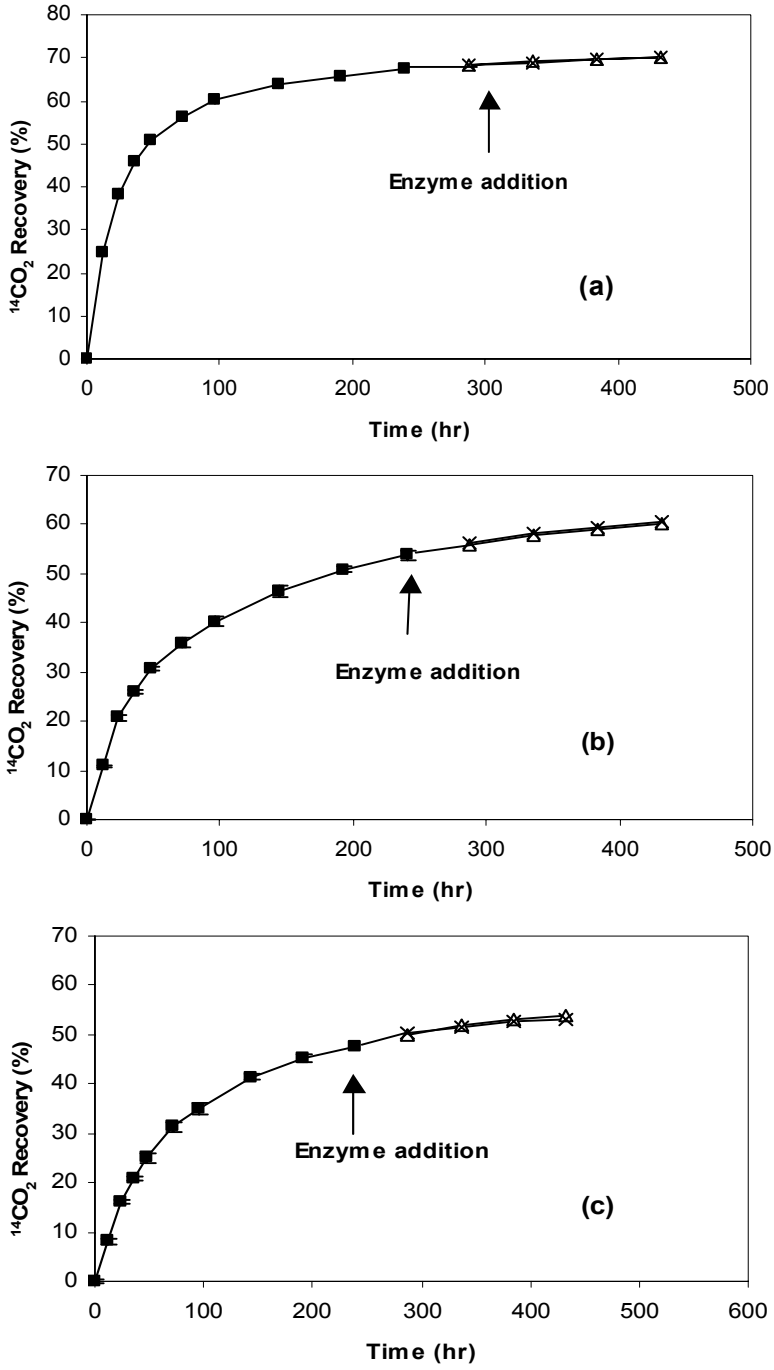


FIGURE S4.7 Effect of cellulase and hemicellulase addition on the bioavailability of toluene sorbed to degraded office paper aged for (a) 1 day, (b) 30 days and (c) 180 days.

Data are presented as the percentage of toluene added initially recovered as $^{14}CO_2$. Data before enzyme addition (■) are averages of four replicates. Data with enzyme addition (Δ) and data without enzyme addition (×) are averages of

duplicate samples. Error bars correspond to standard deviations. Some error bars are too small to view.

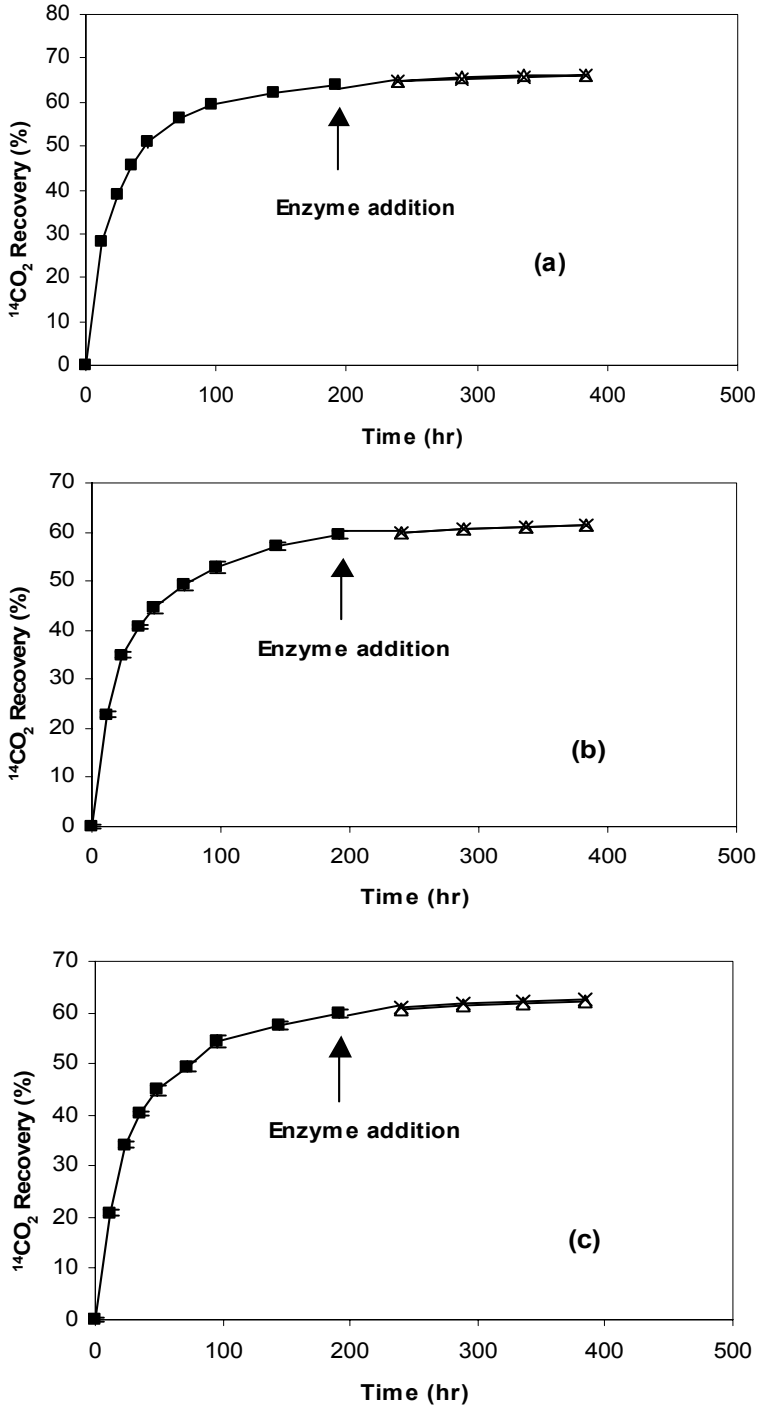


FIGURE S4.8 Effect of cellulase and hemicellulase addition on the bioavailability of toluene sorbed to degraded newsprint aged for (a) 1 day, (b) 30 days and (c) 180 days.

Data are presented as the percentage of toluene added initially recovered as $^{14}CO_2$. Data before enzyme addition (■)

are averages of four replicates. Data with enzyme addition (Δ) and data without enzyme addition (×) are averages of duplicate samples. Error bars correspond to standard deviations. Some error bars are too small to view.

TABLE S4.1 Sorption of Toluene to Newsprint and NP-Lignin after 30 days of aging (%) [a, b]

sorbents	Toluene uptake (%)		K_p (mL/g) [d]
	whole sorbent [c]	lignin extracted from sorbent	
newsprint	46.33 (0.83)	46.08 (1.31)	102.67 (5.39)

[a] Data are the percentage of sorbed toluene based on total toluene added. [b] Data are the averages of duplicate samples and values in parenthesis are the standard deviations. [c] Mass of newsprint is similar to those used in bioavailability tests (3 g). [d] K_p values were calculated for the sorption of toluene by extracted lignin.

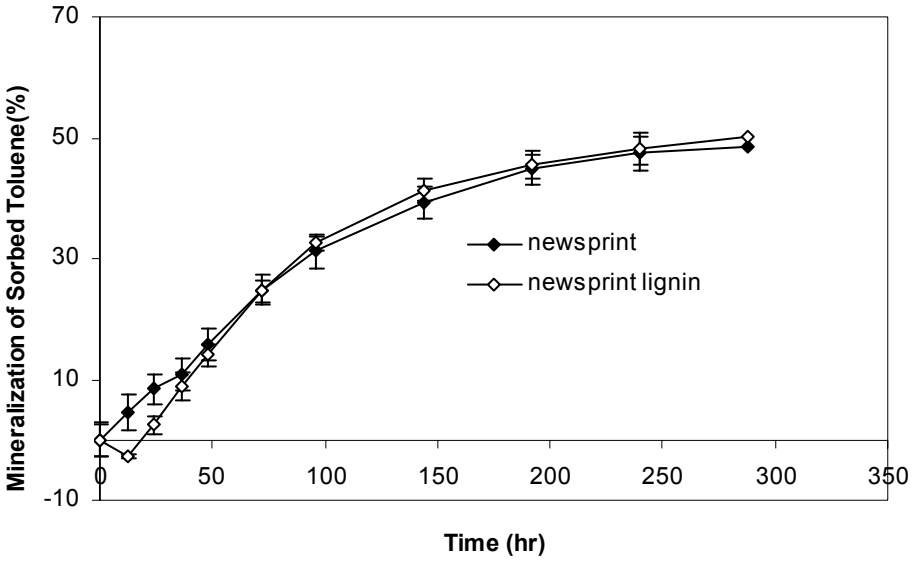


FIGURE S4.9 Bioavailability of toluene aged for 30-d with fresh newsprint and isolated lignin of equivalent mass.

Data were calculated based on equation 1 and data are averages of three replicates. Error bars correspond to standard deviations. Some error bars are too small to view.

Appendices

Appendix A Data of manuscript 1

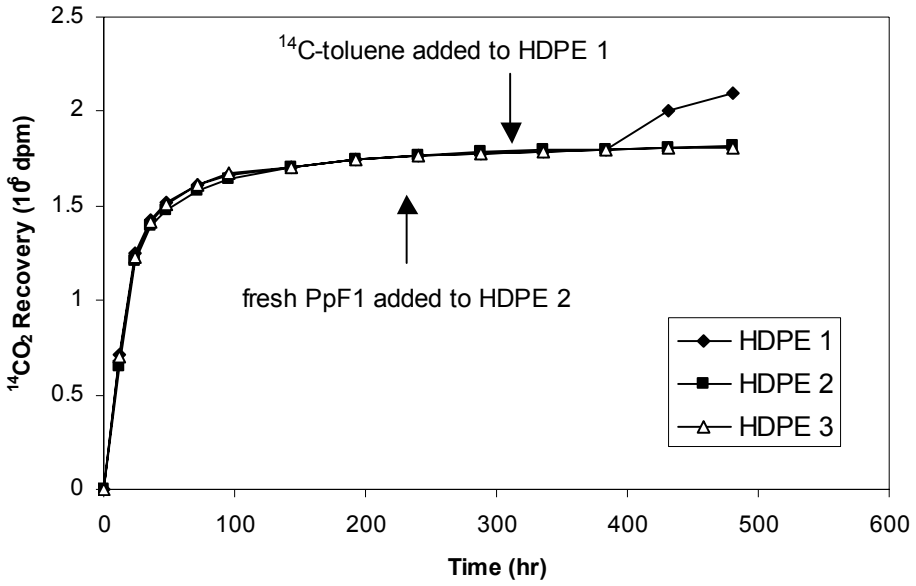


FIGURE A.1 Comparison of bioavailability of toluene sorbed to HDPE under different treatment

TABLE A.1 Measurement of Liquid Phase ^{14}C Concentration by Different Treatments

	treatment 1 [a, b]	treatment 2 [a,c]
sample 1	1810.56	1845.09
sample 2	1814.38	1822.73

[a] Concentrations are in dpm/0.5mL of liquid sample. [b] Treatment 1 is direct injection of samples with cells and particulates to scintillation cocktail. [c] Treatment 2 is the injection of cells and particulates to scintillation cocktail after cell lysis; cell lysis was performed by adding lysozyme followed by alternating freezing and thaw cells with –70°C dry ice and 65°C heat block respectively for three times.

TABLE A.2 Bioavailability, Extraction and Combustion Data (dpm)

PVC 1-d					
Time (hr)	PVC 1	PVC 2	PVC 3	blank 1	blank 2
0	0	0	0	0	0
12	103183	100625	90898.5	120135	123934
24	66177.5	58940.3	59800.3	31203.1	32885.5
36	27822.4	32045.7	33359.5	8472.01	9029.85
48	17638.1	21417	21079	4189.55	4149.52
72	38157.1	40069.8	39426.9	5104	4266.93
96	25632.7	27903	24772.2	3569.08	4691.12
144	26812.4	28013.5	30829.2		
192	20660.6	19371	20098		
240	13077.2	10504.6	15366.05		
312	19557.8	15873.7	20939.3		
360	10044.8	9184.14	9521.45		
408	9500.17	7742.07	8959.56		
456	7653.94	6295.07	8038.6		
504	6263.15	6055.97	6316.98		
after aging	7377.42	6891.2	6761.57	6913.11	7009.39
liquid	1810.56	1814.38	2442.05	6350.1	6165.81
after filtration	369.54	362.52	348.35		
BA 1	6531.97	6192.08	6018.24		
BA 1vol (mL)	12	12	12		
BA 2	3606.99	3683.91	3278.98		
BA 2 vol (mL)	12	12	12		
BA 3	1746.06	1869.64	1739.19		
BA 3 vol (mL)	15	15	15		
BA 4	1936.66	1957.04	1951.02		
BA 4 vol (mL)	15	15	15		
BA 5	1548.89	1447.66	1623.54		
BA 5 vol (mL)	15	15	15		
BA 6	873.23	972.45	1033.99		
BA 6 vol (mL)	15	15	15		
BA 7	523.65	472.06	523.11		
BA 7 vol (mL)	15	15	15		

HDPE 1-d					
Time (hr)	HDPE 1	HDPE 2	HDPE 3	blank 1	blank 2
0	0	0	0	0	0
12	187942	162640	175610	40850.3	40652
24	145368	138912	137948	18774.3	18562.4
36	43617.2	46945	46139.5	6194.89	6108.96
48	21901.9	21205.2	23975.6	2329.87	2437.58
72	24415.5	26484.3	25780.4		
96	13297.8	14872	15922.7		
144	8805.8	13974.5	8622.34		
192	9929.9	11326.3	8657.21		
240	7321.59	8932.75	7729.54		
after aging	3536.43	3618.28	3756.55	3316.18	2952.16
liquid	3673.32	3428.21	3670.52	2798.07	2486.65
after filtration	490.59	472.29	516.39		
BA 1	505.86	579.57	547.68		
BA 1vol (mL)	44.5	42.5	42		
BA 2	385.63	379.96	323.81		
BA 2 vol (mL)	22	21	20		
BA 3	275.63	287.66	294.85		
BA 3 vol (mL)	20	20	20		
BA 4	173.22	166.24	159.63		
BA 4 vol (mL)	20	20	20		
BA 5	111.26	99.32	103.22		
BA 5 vol (mL)	20	20	20		

Office paper 1-d						
Time (hr)	OP 1	OP 2	OP 3	OP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	61213.1	48808.8	48433.4	56396.9	0	0
24	29410.8	37854.2	40060.3	29081.2	47957.4	46743.9
36	20305.6	18644	15897.3	21695.6	28306.9	27154.7
48	13293.4	14865.7	14507.2	11054.9	18069.2	17532.4
72	14530.9	15614.3	14727.9	12059.9	9724.44	11978.1
120	10367.3	10783.3	10398.75	11128.9	9776.89	10015.3
168	7794.21	7865.24	8158.75	9159.1	7940.24	8237.46
216	4709.32	4976.88	6401.06	7979.87	3391.19	3645.46
264	3926.98	4239.21	5529.65	4821.25		
after aging	6241.55	6242.9	6430.09	6304.85	6097.06	6044.75
liquid	1018.33	1126.77			4974.35	4863.23
after filtration	232.52	215.05				
BA 1	218.7	176.97				
BA 1 vol (mL)	21	21.5				
BA 2	1694.45	1824.31				
BA 2 vol (mL)	10	10.5				
BA 3	190.28	224.81				
BA 3 vol (mL)	16.5	17				
BA 4	141.28	186.99				
BA 4 vol (mL)	15	15				
base trap 1	328.91	342.49				
base trap 2	27.59	20.69				
base trap 3	0	0				
base trap 4	0	0				
solid mass (g)	3.8229	3.5381				

Degraded Office paper 1-d						
Time (hr)	DOP 1	DOP 2	DOP 3	DOP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	141146	140055.5	140754	139357	90769.2	93772.6
24	69450.1	78334	79302.5	77365.5	82042.9	83925
36	43930.5	43814.55	44944.3	42684.8	28970.5	20456.7
48	28993.3	27111.35	27079.9	27142.8	13184.5	14653.2
72	30219.5	30612.8	31405.8	29819.8	10423.9	9938.4
120	21271.1	20607.25	20543.8	20670.7	7342.99	6478.51
168	20118	20389.85	20551.5	20228.2	4323.14	4696.7
216	10842.2	12660.65	11658.1	13663.2		
264	8392.46	8969.28	8848.57	9089.99		
after aging	9450.71	9369.81	9397.35	9434.49	9434.45	9443.24
liquid	1858.74	1783.97			8469.28	8423.55
after filtration	409.16	414.55				
BA 1	613.87	625.66		base 1	288.17	302.28
BA 1 vol (mL)	15	16.5		base 1 vol	24	23.5
BA 2	2409.44	2336.95		base 2	230.54	247.66
BA 2 vol (mL)	18.5	17.5		base 2 vol	28	29
BA 3	433.3	419.66		base 3	191.88	210.55
BA 3 vol (mL)	30	29.6		base 3 vol	28	28.5
BA 4	190.48	250.14		base 4	131.56	162.13
BA 4 vol (mL)	22	24		base 4 vol	288.17	302.28
BA 5	106.98	154.41				
BA 5 vol (mL)	20	21.5				
HA	0	0				
HA vol (mL)	0	0				
NH	71.82	83.3				
NH vol (mL)	90	89				
FA	152.16	167.4				
FA vol (mL)	110	120				
base trap 1	354.64	387.73				
base trap 2	5.04	19.22				
base trap 3	0.9	0				
base trap 4	0	0				
solid mass (g)	5.9741	5.8793				

Newsprint 1-d						
Time (hr)	NP 1	NP 2	NP 3	NP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	166631	171183	176012	175487	125099	129978
24	67416.6	60267.8	70660.6	76671.1	56611.6	57680.6
36	35795.1	34738.1	37028.6	31639.2	14269.7	12823.89
48	39486.1	24001	27197.1	23627.5	6659.86	6853.24
72	27118	22740.5	29615.8	24227.2	6443.939	6904.872
120	16498.6	16549.9	19375	14432.2		
168	14010.4	12824.2	13691.4	12158.6		
216	8879.2	8248.95	8623.64	8287.09		
264	5942.15	6237.55	6058.79	6014.77		
after aging	8795.11	8743.03	8843.67	8736.71	9023.72	9110.36
liquid	1335.06	409.16			7963.42	8148.22
after filtration	1451.38	414.55				
BA 1	1232.97	1416.2		base 1	523.85	632.26
BA 1 vol (mL)	18	16.5		base 1 vol	30	30
BA 2	451.5	557.14		base 2	489.65	391.53
BA 2 vol (mL)	27	26.5		base 2 vol	25	24
BA 3	395.97	331.86		base 3	410.39	304.88
BA 3 vol (mL)	25.5	25		base 3 vol	26	24
BA 4	293.55	284.75		base 4	223.12	198.69
BA 4 vol (mL)	25.5	24.5		base 4 vol	25.5	26
HA	1214.2	1232.95				
HA vol (mL)	15	15				
NH	138.35	127.19				
NH vol (mL)	104	102				
FA	160.09	179.22				
FA vol (mL)	92	92				
base trap 1	141.53	193.65				
base trap 2	70.49	78.94				
base trap 3	0.61	2.81				
base trap 4	0	0				
solid mass (g)	5.7102	5.5027				

Degraded newsprint 1-d						
Time (hr)	DNP 1	DNP 2	DNP 3	DNP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	158963	166895	150594	158744.5	120096	128125.2
24	66373.7	55863.3	68101.2	62983	55951.3	47505.4
36	34408.6	41392	38273.9	39895	15596.4	16243.52
48	30008	27100.3	28706.9	27913	5830.94	5158.3
72	30566.5	33988.9	31445.3	32767	8624.39	8924.23
120	15108.8	17182.84	16308	19745		
168	13700.4	18399.6	14306	16753		
216	9177.33	11841.9	9540.81	10791		
after aging	8014.79	7907.26	7947.3	8234.49	8099.53	8103.62
liquid	2260.67	2295.38			7211.42	7321.96
after filtration	637.31	552.07				
BA 1	980.52	717.14		base 1	843.12	795.94
BA 1 vol (mL)	3	5		base 1 vol	19.5	19.5
BA 2	2090.08	1796.82		base 2	617.06	755.86
BA 2 vol (mL)	19	22		base 2 vol	20.5	21
BA 3	1564.92	1760.65		base 3	765.2	662.97
BA 3 vol (mL)	20	19.5		base 3 vol	20	21.5
BA 4	649	765.14		base 4	342.1	389.27
BA 4 vol (mL)	16	15.5		base 4 vol	19.5	19
BA 5	205.24	207.67				
BA 5 vol (mL)	22	19.9				
BA 6	151.22	162.47				
BA 6 vol (mL)	23.1	22.5				
HA	2543.7	1947.78				
HA vol (mL)	15	15				
NH	57.34	43.89				
NH vol (mL)	79	88				
FA	182.75	146.13				
FA vol (mL)	75	105				
base trap 1	226.58	239.1				
base trap 2	26.1	10.97				
base trap 3	5.61	3.73				
base trap 4	1.7	2.27				
solid mass (g)	8.8577	8.4176				

Rabbit food 1-d							
Time (hr)	RF 1	RF 2	RF 3		blank 1	blank 2	
0	0	0	0		0	0	
12	116765	113794	116147		129016.7	125930.3	
24	114615	120268	105533.4		50888.6	52912.8	
36	38903.1	42828.6	43220.1		14750.7	16424.7	
48	21777.4	27961.2	28545.6		13445.5	12821.5	
72	20677.2	15146.9	20082.8		7732.63	7859.42	
120	13105	12178.4	15083.03		6661.15	7137.62	
168	10075.2	10023.9	10883.2				
216	8752.31	7951.41	8972.1				
264	5326.11	4948.54	5113.43				
after aging	9542.92	9204	9231.51		9229.93	9215.51	
liquid	2630.76	2751.04	2480.15		7965.75	8010.78	
after filtration	715.16	954.19	736.265				
BA 1	1320.23	1537.74	1598.7	base 1	758.88	929.43	771.28
BA 1 vol (mL)	22	23	23	base 1 v	25	25	25
BA 2	993.81	1121.41	750.38	base 2	1007.31	1357.86	1360.17
BA 2 vol (mL)	15	15	15	base 2 v	20	20	20
BA 3	556.16	573.09	384.72	base 3	617.1	867.91	551.51
BA 3 vol (mL)	15	15	15	base 3 v	22	20	20
BA 4	363.03	400.1	386.96	base 4	981	952.8	921.09
BA 4 vol (mL)	15	15	15	base 4 v	22	18.5	19.5
BA 5	237.93	249.55	247.84	base 5	413.9	631.34	358.51
BA 5 vol (mL)	15	15	15	base 5 v	22	18.5	19.5
HA	1668.55	1653.975	1929.48				
HA vol (mL)	15	15	15				
NH	222.55	210.56	213.09				
NH vol (mL)	153	121	103				
FA	158.73	111.66	123.81				
FA vol (mL)	158	152	157				
base trap 1	532.47	532.36	614.08				
base trap 2	136.03	92.31	264.67				
base trap 3	2.02	0.48	15.97				
base trap 4	1.26	0	4.78				

Degraded Rabbit food 1-d							
Time (hr)	DRF 1	DRF 2	DRF 3		blank 1	blank 2	
0	0	0	0		0	0	
12	138390	131339	131298		125099	129978	
24	86193.8	101180	96594.1		56611.6	47680.6	
36	57578.6	49508.2	55040.2		14590.37	18224.43	
48	30280.2	35905	32961.1		13299.35	13671.27	
72	22033.4	22729.3	22957.6		7648.58	7773.992	
120	12980.7	13121.4	13398.7		6588.746	7060.037	
168	13585.3	12484.6	13393.1				
216	10328.6	9924.35	10439.6				
264	7896.07	8102.86	8527.98				
	5532.17	6102.67	6023.52				
	4103.98	4987.63	5010.75				
after aging	9132.18	9247.82	9035.289		9051.99	9085.36	
liquid	3014.42	2822.35	2918.385		7936.12	8041.48	
after filtration	791.44	824.99	808.215				
BA 1	2066.75	2026.91	2046.81	base 1	442.18	405.41	438.29
BA 1 vol (mL)	15	15	15	base 1 v	20	20	20
BA 2	322.75	373.31	323.81	base 2	384.94	482.14	429.37
BA 2 vol (mL)	20	20	20	base 2 v	20	20	20
BA 3	275.63	287.66	294.85	base 3	524.55	486.63	513.35
BA 3 vol (mL)	20	20	20	base 3 v	20	20	20
BA 4	225.35	237	241.98	base 4	240.26	224.88	225.18
BA 4 vol (mL)	20	20	20	base 4 v	19	20	19.5
BA 5	177.87	180.74	170.12				
BA 5 vol (mL)	20	20	20				
HA	272.04	240.14	256.98				
HA vol (mL)	15	15	15				
NH	117.29	142.78	113.09				
NH vol (mL)	104	98	103				
FA	76.94	79.2	76.81				
FA vol (mL)	158	159	157				
base trap 1	372.71	438.77	464.59				
base trap 2	38.67	112.01	121.59				
base trap 3	5.13	5.2	0				
base trap 4	1.38	1.59	0				

PVC 30-d					
Time (hr)	PVC 1	PVC 2	PVC 3	blank 1	blank 2
0	0	0	0	0	0
12	39459	35969.8	36671.5	24566.3	25681.4
24	35457.7	34719.6	34189.5	11012.7	11455.7
36	21067.6	22207.7	22325.9	3869.67	2861.73
48	18532.6	16995.2	18182.6	1583.37	1407.68
72	23540.6	26267.8	23558.1	1507.81	1617.05
96	22683.7	28315.6	22464.8		
144	32246.3	38086.6	34237.5		
192	32647.6	32116.3	32092.1		
240	23693.2	23828	23675		
312	29440.7	30280.6	28708		
360	18594.8	16806.5	18073.1		
408	15850.1	14703.1	15849.2		
456	13427	12574.8	12931.9		
504	8726.9	8236.4	8833		
after aging	1615.94	1466.71	1603.47	1581.42	1602.87
liquid	1358.47	1440.48	1423.66	1418.06	1416.21
after filtration	292.3	350.83	342.1		
BA 1	11979.52	11518	12120		
BA 1vol (mL)	15	15	15		
BA 2	11813.4	12867.5	10697.2		
BA 2 vol (mL)	12	12	12		
BA 3	6136.62	5633.25	5732.69		
BA 3 vol (mL)	12	12	12		
BA 4	2577.53	2150.09	2442.11		
BA 4 vol (mL)	12	12	12		
BA 5	1003.59	1069.89	1158.79		
BA 5 vol (mL)	10.5	11	10.7		
BA 6	120.53	113.29	111.98		
BA 6 vol (mL)	10	12.5	14		

HDPE 30-d					
Time (hr)	HDPE 1	HDPE 2	HDPE 3	blank 1	blank 2
0	0	0	0	0	0
12	189653	155073	179172	41692.3	37892.3
24	135870	169331	139191	18750.3	18912.4
36	30682.1	27593.5	28083.2	6123.47	6020.5
48	32232.5	31427.6	32137.5	2134.59	2437.88
72	23308	24420.4	23913		
96	11654	12210.2	11235.6		
144	12752.7	11610.2	10727.6		
192	6388.41	5803.92	6060.33		
240	5329.61	5923.11	5211.22		
after aging	3476.11	3799.39	3665.89	3267.91	3032.98
liquid	4655.42	3929.8	4370.52	2748.09	2533.76
after filtration	654.85	631.96	646.33		
BA 1	2237.71	2297.58	2247.68		
BA 1vol (mL)	13	12	12.8		
BA 2	447.89	497.55	501.26		
BA 2 vol (mL)	14	15	16		
BA 3	103.69	111.89	177.98		
BA 3 vol (mL)	14.5	15.9	15.6		

Office paper 30-d						
Time (hr)	OP 1	OP 2	OP 3	OP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	19961.6	19387.1	19782.4	19299.3	21074.9	25694.2
24	15676.2	16098.2	15478	14328.7	30468	32331.4
36	8658.26	9145.39	9239.87	9397.44	17709.2	18395.7
48	8330.45	8664.61	12889	10237.2	9268.25	10469.2
72	18936.7	12861.6	13920.6	13889.6	7741.97	10064.7
120	12118	12551.5	12228.2	12289.7	7106.85	6467.15
168	18429.8	17970.2	15416.8	16852.9	7503.23	5465.77
216	10866.7	13455	13860.4	13354.1	2490.38	2437.65
264	13885	12508.8	15314.8	14523.6		
after aging	5976.4	5656.75	5919.77	5743.08	5997.06	5844.75
liquid	1428.72	1437.27			4674.35	4963.23
after filtration	517.9	522.79				
BA 1	364.11	379.55		base 1	523.64	506.23
BA 1 vol (mL)	15	14.7		base 1 v	25	25.5
BA 2	2074.54	1994.23		base 2	358.44	323.52
BA 2 vol (mL)	15.5	14.5		base 2 v	25	24
BA 3	112.46	126.57		base 3	223.87	215.89
BA 3 vol (mL)	25	22.5		base 3 v	25	25
base trap 1	390.95	401.88				
base trap 2	42.65	32.76				
base trap 3	0	0				
base trap 4	0	0				
solid mass (g)	5.754	5.7124				

Degraded Office paper 30-d						
Time (hr)	DOP 1	DOP 2	DOP 3	DOP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	65091.1	60737.4	57844.2	139357	68557.1	70399.7
24	52181.3	58331.9	59390.8	77365.5	73130.4	70724.2
36	29784.8	31498.9	24941.7	42684.8	29990.6	25525.4
48	25870.3	23041.7	32428.7	27142.8	10434.8	12096.3
72	29821.6	29786.1	29999.6	29819.8	6072.47	9460.44
120	24644.8	24912.6	23782.7	20670.7	4840.51	2716.12
168	33660.9	36450.5	31988	20228.2		
216	26609.5	27254	21904.6	13663.2		
264	15635.1	13442.2	20211.4	9089.99		
after aging	8959.43	8941.2	8726.13	8875.58	8734.01	8732.55
liquid	3055.97	2860.1			7789.25	7713.5
after filtration	1724.94	1634.25				
BA 1	863.24	927.46		base 1	777.44	628.28
BA 1 vol (mL)	30	29		base 1 vol	29	31
BA 2	5566.24	4705.61		base 2	234.38	454.37
BA 2 vol (mL)	8.5	9.5		base 2 vol	29	29.5
BA 3	460.65	342.3		base 3	221.17	187.89
BA 3 vol (mL)	24	24.6		base 3 vol	29	29
BA 4	279.79	223.97		base 4	157.95	144.21
BA 4 vol (mL)	23	20.5		base 4 vol	29	30.5
BA 5	177.59	189.42				
BA 5 vol (mL)	20.5	21.1				
HA	0	0				
HA vol (mL)	0	0				
NH	166.81	111.38				
NH vol (mL)	130	120				
FA	228	210.71				
FA vol (mL)	105	95				
base trap 1	29	30.5				
base trap 2	532.89	548.16				
base trap 3	53.31	67.21				
base trap 4	2.11	7.79				
solid mass (g)	1.54	0				

Newsprint 30-d						
Time (hr)	NP 1	NP 2	NP 3	NP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	127907	135533	145491	137372	125099	129978
24	63535.3	65535.6	64545.7	58349.1	56611.6	57680.6
36	29663.9	26156.6	26389.4	27976.7	14269.7	12823.89
48	22570.8	23809.1	22608.6	20101.1	6659.86	6853.24
72	29296.8	28519.8	27895.5	27320.1	6443.939	6904.872
120	18633.6	18117	17583.4	17631.5		
168	22158.8	23011.9	23322	21950.3		
216	15568	15391.9	14506.2	14654.8		
264	6600.63	7798.71	6704.03	8359.83		
after aging	8262.13	8015.64	8090.53	8139.91	8124.13	8208.12
liquid	1972.12	1888.96			7089.25	7013.5
after filtration	909.96	927.47				
BA 1	498.7	487.95		base 1	665.22	721.97
BA 1 vol (mL)	15	15.5		base 1 vol	23.5	21.5
BA 2	1742.69	2077.85		base 2	539.21	482.85
BA 2 vol (mL)	17.5	16.5		base 2 vol	26.5	27.5
BA 3	357.38	343.78		base 3	383.64	397.61
BA 3 vol (mL)	25	25.5		base 3 vol	22.5	24
BA 4	296.22	287.39		base 4	237.63	241.95
BA 4 vol (mL)	26	25.2		base 4 vol	21	24.5
HA	1687.38	2167.01				
HA vol (mL)	15	15				
NH	139.44	143.21				
NH vol (mL)	93	82				
FA	208.05	237.4				
FA vol (mL)	105	98				
base trap 1	180.62	226.96				
base trap 2	96.26	101.54				
base trap 3	12.56	7.51				
base trap 4	1.29	1.02				
solid mass (g)	8.0917	8.7153				

Degraded newsprint 30-d						
Time (hr)	DNP 1	DNP 2	DNP 3	DNP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	130222	132114	130189	131168	132219	124985
24	67050.36	67146.21	46688.11	67098.29	48578.9	43655.1
36	32042.6	34882	30242.1	33462.3	13162.7	12478.6
48	22984.5	22680.3	22370	22832.4	6024.17	6942.59
72	25213.4	27778.8	25553.1	26496.1	6414.22	6488.87
120	20505.8	21103.8	21507.2	20804.8	3660.49	4238.34
168	24728.7	22472.7	24507.6	23600.7		
216	13828.5	12923.4	12819.7	13375.8		
after aging	7881.49	7644.72	7974.3	7833.53	7964.54	7804.44
liquid	2118.46	2244.1			7098.28	7090.86
after filtration	562.32	567.38				
BA 1	1173.01	1285.46		base 1	608.3	763.88
BA 1 vol (mL)	9	10		base 1 vol	18	16
BA 2	2011.84	2244.88		base 2	641.22	705.55
BA 2 vol (mL)	25	25		base 2 vol	18	19.5
BA 3	1050.44	935.45		base 3	359.55	627.29
BA 3 vol (mL)	26	25		base 3 vol	20	19.5
BA 4	257.09	312.92		base 4	409.23	555.32
BA 4 vol (mL)	19	20		base 4 vol	21	21.5
BA 5	197.83	172.09		base 5	309.21	284.13
BA 5 vol (mL)	21.5	22.5		base 5 vol	21	21.5
HA	2289.49	2699.41				
HA vol (mL)	15	15				
NH	47.76	65.09				
NH vol (mL)	78	78				
FA	142.58	343.57				
FA vol (mL)	105	92				
base trap 1	199.61	216.55				
base trap 2	101	56.69				
base trap 3	2.83	0.83				
base trap 4	0	0				
solid mass (g)	8.8001	8.9595				

Degraded Rabbit food 30-d							
Time (hr)	DRF 1	DRF 2			blank 1	blank 2	
0	0	0			0	0	
12	62678.5	72528.2			129016.7	115930.3	
24	118121	124427			50888.6	57912.8	
36	74303.5	57947.9			27230.7	23044.8	
48	44362.7	33915.1			17452.9	16300.4	
72	23340.1	15401.6			6549.6	6828.04	
96	8504.11	11653.1					
144	7997.95	10992.7					
192	15235.2	14985.4					
240	7079.64	7689.41					
288	5252.69	5862.14					
336	4013.25	3994.12					
after aging	9173.97	9104.3			9051.99	9085.36	
liquid	2807.73	2822.35			8076.12	7941.48	
after filtration	877.42	873.75					
BA 1	1451.67	1211.45		base 1	617.3	610.14	
BA 1 vol (mL)	22	23		base 1 v	28	29.5	
BA 2	689.21	644.28		base 2	617.3	610.14	
BA 2 vol (mL)	20	20.5		base 2 v	28	29.5	
BA 3	448.52	465.21		base 3	215.94	225.18	
BA 3 vol (mL)	20	20.5		base 3 v	30	30	
BA 4	240.89	177.73					
BA 4 vol (mL)	17	18					
HA	1803.3	1898.55					
HA vol (mL)	15	15					
NH	113.63	141.09					
NH vol (mL)	98	78					
FA	103.26	99.2					
FA vol (mL)	135	113					
base trap 1	303.57	397.51					
base trap 2	246.03	255.92					
base trap 3	108.53	14.03					
base trap 4	0	1.03					

PVC 180-d					
Time (hr)	PVC 1	PVC 2	PVC 3	blank 1	blank 2
0	0	0	0	0	0
12	36531	32166	33546	24566.3	25681.4
24	22254.1	22820.5	22013.6	11012.7	11455.7
36	31379.4	26098	27099	3869.67	2861.73
48	14058.9	14797.7	14009.6	1583.37	1407.68
72	25007.3	26680.4	26030.5	1507.81	1617.05
96	19752.2	22897.5	21947.1		
144	32472.5	33137.9	32865.9		
192	32277.1	35029.3	32426.5		
240	23471.1	23493.9	23526.9		
312	20259	21720.6	20856.3		
360	15213.6	16152.3	14589.9		
408	12964.1	14466	14731.4		
456	14479.2	15663.2	15201.3		
504	10640.1	11530.1	11125.6		
after aging	1278.05	1221.62	1233.47	1581.42	1602.87
liquid	1528.59	1575.8	1423.66	1418.06	1416.21
after filtration	456.18	514.18	342.1		
BA 1	17097.7	16649.2	17293.1		
BA 1vol (mL)	10.5	10	10		
BA 2	10037.08	12699.03	11124.69		
BA 2 vol (mL)	10	10	11		
BA 3	6264.9	6875.23	6438.29		
BA 3 vol (mL)	12	12	12		
BA 4	3131.98	3435.84	3498.45		
BA 4 vol (mL)	12	12	12		
BA 5	3759.43	3658.46	3515.33		
BA 5 vol (mL)	10.5	11	10.7		
BA 6	1254.33	1564.32	1137.29		
BA 6 vol (mL)	10	11	10.5		
BA 7	969.43	875.24	961.28		
BA 7 vol (mL)	10	10	10		
BA 8	223.98	214.89	311.66		
BA 8 vol (mL)	10.5	11	10.5		

HDPE 180-d					
Time (hr)	HDPE 1	HDPE 2	HDPE 3	blank 1	blank 2
0	0	0	0	0	0
12	199995	194534	196021	40850.3	36652
24	130438	121577	123434	18774.3	18562.4
36	29753.9	31182.1	30837.9	6194.89	6108.96
48	12892.2	12510.8	13003.5	3424.98	3125.54
72	22550.8	23374.6	23835.5		
96	10265.93	11517.28	11965.41		
144	10667.2	11031.41	11327.9		
192	7952.31	7741.21	7512.22		
240	5386.52	5412.23	5874.98		
after aging	3558.42	3749.92	3639.219	3365.66	3223.76
liquid	4204.6	5421.23	4651.29	2746.34	2864.99
after filtration	829.51	801.3	858.93		
BA 1	1081.96	1210.95	1217.24		
BA 1vol (mL)	13	12	12.8		
BA 2	245.5	235.89	275.98		
BA 2 vol (mL)	14	15	16		
BA 3	103.69	111.89	177.98		
BA 3 vol (mL)	14.5	15.9	15.6		

Office paper 180-d						
Time (hr)	OP 1	OP 2	OP 3	OP 4	blank 1	blank 2
0	0	0	0	0		
12	19046.3	17344	19202.7	19870.3		
24	16858.3	12802.6	15403.7	13419.8		
36	11890.3	9698.42	9868.95	9874.91		
48	7846.41	7446.3	8131.03	7428.51		
72	11846.8	12378.7	9816.06	10672.9		
120	8480.82	9118.61	9073.04	8191.54		
168	10376	12490.7	10243.8	10205.9		
216	7596.32	9004.12	7512.12	7516.8		
264	7424.09	8504.96	7842.96	7007.46		
after aging	5410.94	5548.13	5673.04	5467.45	5416.18	5552.17
liquid	2375.08	2452.98			4398.07	4486.65
after filtration	1625.67	1218.43				
BA 1	2370.98	2456.17		base 1	540.03	464.89
BA 1 vol (mL)	7	7		base 1 v	22	23
BA 2	281.63	378.56		base 2	199.17	284.2
BA 2 vol (mL)	12	13		base 2 v	20	20
BA 3	447.89	497.55		base 3	265.98	210.62
BA 3 vol (mL)	14	15		base 3 v	20	20
BA 4	103.69	111.89		base 4	227.51	176.57
BA 4 vol (mL)	14.5	15.9		base 4 v	19	20
BA 5	218.97	195.12				
BA 5 vol (mL)	25	23				
BA 6	154.23	172.12				
BA 6 vol (mL)	22	19				
HA	0	0				
HA vol (mL)	0	0				
NH	116.66	104.21				
NH vol (mL)	93	97				
FA	178.51	197.41				
FA vol (mL)	75	80				
base trap 1	418.18	356.22				
base trap 2	117.78	138.98				
base trap 3	13.33	10.33				
base trap 4	3.97	0				
solid mass (g)	5.1039	4.9842				

Degraded Office paper 180-d						
Time (hr)	DOP 1	DOP 2	DOP 3	DOP 4	blank 1	blank 2
0	0	0	0	0		
12	43762.3	45936.6	47595.7	50736.6		
24	38435.9	47418.1	49149.5	45285.9		
36	24317.6	28145.4	27064.6	22571		
48	23929	25549.5	23549.5	29836.2		
72	35001.8	37892.4	34628.6	31761.3		
120	20327.4	22405.9	19017.9	23476.1		
168	39909.6	29901.9	38237.2	27560.2		
216	22003.2	22881.3	19501.8	26067.6		
264	13565.4	13271.3	12058	11770.1		
after aging	8784.82	8822.13	8697.89	8768.28	8828.41	8776.34
liquid	3870.08	3820.17			7764.37	7859.33
after filtration	2988.74	3000.47				
BA 1	4149.29	4137.34		base 1	630.43	648.65
BA 1 vol (mL)	15	16.5		base 1 vol	22	23
BA 2	746.2	752.44		base 2	310.83	176.68
BA 2 vol (mL)	9.5	10.5		base 2 vol	21	20
BA 3	538.28	468.93		base 3	139.11	196.67
BA 3 vol (mL)	20	20		base 3 vol	20	20
BA 4	300.4	281.07		base 4	98.75	88.24
BA 4 vol (mL)	21	22		base 4 vol	19	20
BA 5	245.55	243.56				
BA 5 vol (mL)	23	24.5				
BA 6	197.53	184.12				
BA 6 vol (mL)	25	23				
HA	0	0				
HA vol (mL)	0	0				
NH	116.66	104.21				
NH vol (mL)	93	97				
FA	178.51	197.41				
FA vol (mL)	75	80				
base trap 1	601.38	597.3				
base trap 2	66.1	57.41				
base trap 3	8.57	17.27				
base trap 4	0.68	0				
solid mass (g)	5.5637	5.8742				

Newsprint 180-d						
Time (hr)	NP 1	NP 2	NP 3	NP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	130232	120095	116616	111668	123934	124125
24	73736.2	68028.6	80480.2	69841.2	51362.6	52358.7
36	29177.8	31001.2	34224.7	35308.9	23137.9	18150.9
48	16744.8	23848.8	23564.2	23533.9	6515.95	5430.58
72	26663.2	36742.2	35941.7	33604.2	4047.64	4075.23
120	16686.1	14185.9	20757.8	18306.6		
168	20059.9	15979.8	21956.1	20368.3		
216	10684.5	9051.92	11444.6	14733.6		
264	4562.46	4301.65	7728.76	8744.51		
after aging	8246.4	7978.15	8030.1	8084.88	8006.29	7087.65
liquid	2534.69	2527.46			8116.35	6998.65
after filtration	1457.85	1466.69				
BA 1	1197.5	1199.24		base 1	715.37	655.62
BA 1 vol (mL)	17	19		base 1 vol	20	21
BA 2	496.46	512.18		base 2	447.86	421.41
BA 2 vol (mL)	14	15		base 2 vol	22	20.5
BA 3	469.13	504.21		base 3	614.11	636.14
BA 3 vol (mL)	18	18		base 3 vol	20	20
BA 4	264.55	262.83		base 4	494.15	468.26
BA 4 vol (mL)	20	21		base 4vol	19	22
BA 5	204.87	199.75		base 5	332.15	351.29
BA 5 vol (mL)	22	21		base 5 vol	21	20
BA 6	124.85	137.96		base 6	197.26	156.42
BA 6 vol (mL)	19.5	22		base 6 vol	21	20
HA	1588.41	1637.86				
HA vol (mL)	15	15				
NH	103.76	129.7				
NH vol (mL)	138	142				
FA	141.16	139.92				
FA vol (mL)	202	197				
base trap 1	329.84	316.33				
base trap 2	107.98	229.28				
base trap 3	6.44	15.87				
base trap 4	0.12	0				
solid mass (g)	6.512	6.2932				

Degraded newsprint 180-d						
Time (hr)	DNP 1	DNP 2	DNP 3	DNP 4	blank 1	blank 2
0	0	0	0	0	0	0
12	118885	116990	125639	113978	115397	120360
24	73138	75612	76617	77846	37829.3	42658.4
36	34754.2	34794.2	37413.1	36571.2	12384.9	16587.2
48	23226.4	25979.8	24306.8	28936.2	6489.62	5215.41
72	26527.4	26888.7	24353.4	25103.2	4029.12	4983.41
120	26887.5	30990	26637.3	26784.2	3716.52	2488.79
168	18373.4	20052.5	16939.7	15423.6		
216	14858.3	16315.2	10932	11203.2		
after aging	7738.9	7350	7857.95	7797.6	7630.22	7845.66
liquid	1569.4	1717.35			6845.21	6968.45
after filtration	512.81	475.99				
BA 1	2117.66	2004.03		base 1	453.44	388.97
BA 1 vol (mL)	15	15		base 1 vol	19	22
BA 2	2796.29	2326.58		base 2	637.24	598.46
BA 2 vol (mL)	10	10		base 2 vol	19	20
BA 3	1470.66	1276.74		base 3	359.69	401.2
BA 3 vol (mL)	15	16		base 3 vol	20	21
BA 4	731.66	612.21		base 4	227.89	201.33
BA 4 vol (mL)	20	20		base 4 vol	21	20.5
BA 5	372.56	447.16		base 5	129.88	103.56
BA 5 vol (mL)	20	20		base 5 vol	20	21
BA 6	237.97	289.21				
BA 6 vol (mL)	20	20				
BA 7	157.98	189.54				
BA 7 vol (mL)	20	19				
HA	1567.41	1632.11				
HA vol (mL)	15	15				
NH	106.23	114.66				
NH vol (mL)	128	130				
FA	194.42	201.33				
FA vol (mL)	152	154				
base trap 1	237	230.7				
base trap 2	149.2	110				
base trap 3	39.7	9.87				
base trap 4	25.1	1.51				
solid mass (g)	8.704	10.2388				

Degraded Rabbit food 180-d							
Time (hr)	DRF 1	DRF 2	DRF 3		blank 1	blank 2	
0	0	0	0		0	0	
12	40972.4	43261.8	40426.8		129016.7	115930.3	
24	64934.1	65776.7	69457.2		50888.6	57912.8	
36	69641.7	62942.7	65471.7		27230.7	23044.8	
48	48094.3	49935	47866.7		17452.9	16300.4	
72	38896.5	38672.9	41974		6549.6	6828.04	
96	22557.2	27105.4	23608.4				
144	16676.7	17956.18	19723.9				
192	20679.7	17741.2	19622.8				
240	13840.1	13087.1	12500.2				
288	8138.78	8328.73	8338.49				
336	6329.41	6221.43	6425.89				
after aging	8866.02	9204.12	9282.65		9034.19	9124.57	
liquid	3625.59	3766.22	3633.98		7928.07	8186.65	
after filtration	1098.5	1110.22	858.93				
BA 1	1031.36	993.42	1265.22	base 1	636.11	720.03	719.23
BA 1 vol (mL)	18.5	20.5	19	base 1 v	25	25	25
BA 2	344.79	359.36	341.28	base 2	368.97	491.1	445.89
BA 2 vol (mL)	16	15	16	base 2 v	25	25	25
BA 3	181.14	187.01	197.41	base 3	263.13	230.08	252.79
BA 3 vol (mL)	25	25	25	base 3 v	25	24.5	23.5
BA 4	121.89	148.87	175.49	base 4	179.83	108.85	152.13
BA 4 vol (mL)	22	23	24	base 4 v	25	25	25
HA	510.22	482.13					
HA vol (mL)	15	15					
NH	176.57	242.82					
NH vol (mL)	118	109					
FA	285.79	191.87					
FA vol (mL)	110	98					
		base vol		base vol			
base trap 1	550.3	23	722.69	15			
base trap 2	95.96	16	146.07	15			
base trap 3	22.26	16	13.39	15			
base trap 4	30.11	12	0	15			

Appendix B Data of manuscript 2

TABLE B.1 Enzymatic Hydrolysis Data (dpm)

office paper 1-d				
Time (hr)	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
336	1922.13	1968.53	820.88	1752.44
360	1305.77	1214.83	1069.44	1268.99
408	1159.57	1094.91	1117.81	1532.11
456	1086.9	1686.79	2332.83	1508.87
office paper 30-d				
Time (hr)	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
336	2338.9	2798.86	3173.25	3021.56
360	1507.21	1439.21	1128.3	1143.56
408	2025.66	2187.21	2152.36	2310.33
456	1969.33	2354.2	2509.88	2140.11
office paper 180-d				
Time (hr)	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
336	4648.21	4023.1	5126.84	4986.52
360	3974.89	4016.32	3395.69	3400.18
408	2844.65	2856.21	1875.56	1572.41
456	2013.56	2451.88	1486.75	1754.23
Degraded office paper 1-d	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
288	6111.99	5424.99	3710.93	3917.72
336	3319.2	3564.98	4928.37	4410.39
384	2128.84	3324.68	4136.53	3916.2
432	2037.21	1998.06	2839.64	2976.55
Degraded office paper 30-d	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
288	13213.2	11284.2	15324.1	12235.7
336	10332.5	10652.6	11584.5	10238.8
384	7449.38	8383.99	7560.11	8673.29
432	5229.45	4309.67	4490.15	5239.57
Degraded office paper 30-d	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
288	13829.9	14079.2	14322.1	17165
336	9063.23	10090.2	8821.6	4792.11
384	7793.5	9265.88	7188.37	3546.43
432	2591.68	4294.73	3466.19	1437.17

newsprint 1-d				
Time (hr)	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
288	2562.45	2577.61	7496.16	7045.65
336	4889.71	4802.48	5231.83	5528.65
384	4547.2	4194.82	3856.23	3815.66
432	2348.37	2633.63	2632	1765.66
newsprint 30-d				
Time (hr)	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
288	6488.91	6477.71	3697.3	2564.1
336	2541.483	2452.48	3363.97	2799.39
384	1971.48	2295.38	3044.01	2190.97
432	1936.04	2162.47	1853.38	1764.54
newsprint 180-d				
Time (hr)	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
288	3518.55	2829.28	4896.98	2101.62
336	2108.34	1556.79	3823.35	3994.32
384	1506.13	1117.11	1155.3	1375.2
432	701.19	465.96	431.55	471.33
Degraded newsprint 1-d	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
240	4125.84	5819.98	4956.9	5328.79
288	3044.01	4456.27	2383.07	3378.09
336	2342.16	2765.18	2028.31	2139.04
384	2119.58	2140.39	2208.18	2020.71
Degraded newsprint 30-d	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
240	6471.45	6636.87	6904.56	6554.16
288	3424.71	3023.55	3369.87	3224.13
336	2433.67	2874.3	2566.34	2532.09
384	2009.21	1919.34	1876.39	1965.43
Degraded newsprint 30-d	w/enzyme 1	w/enzyme 2	w/o enzyme 1	w/o enzyme 2
240	6231.3	6345.3	9329.22	6563.4
288	3129	2939.87	3256.86	3108.577
336	3129.45	2847.65	2712.32	2626.13
384	2110.35	1985.63	1954.21	1974.12

TABLE B.2 Bioavailability of Toluene Sorbed to Sorbent Composite and Isolated Lignin (dpm)

Newsprint 1-d							
Time (hr)	NP 1	NP 2	NP 3	NP 4	NP lignin 1	NP lignin 2	NP lignin 3
0	0	0	0	0	0	0	0
12	166631	171183	176012	175487	195262	192358	185969
24	67416.6	60267.8	70660.6	76671.1	52398.5	53682.1	52566.6
36	35795.1	34738.1	37028.6	31639.2	28958.1	28995.4	26884.1
48	39486.1	24001	27197.1	23627.5	24482	26629.7	27078.6
72	27118	22740.5	29615.8	24227.2	24290.5	24060	33008.4
96	16498.6	16549.9	19375	14432.2	13795.9	13108.3	15880.2
144	14010.4	12824.2	13691.4	12158.6	12832.8	12790.1	16590.4
192	8879.2	8248.95	8623.64	8287.09	7475.96	7875.98	8742.67
240	5942.15	6237.55	6058.79	6014.77	5894.23	5949.57	6014.96
Degraded newsprint 1-d							
	DNP 1	DNP 2	DNP 3	DNP 4	DNP lignin 1	DNP lignin 2	
0	0	0	0	0	0	0	
12	158963	166895	150594	158744.5	166976	179270	
24	66373.7	55863.3	68101.2	62983	55626	51617.3	
36	34408.6	41392	38273.9	39895	38494.2	23655.2	
48	30008	27100.3	28706.9	27913	19956.1	19909.8	
72	30566.5	33988.9	31445.3	32767	35828.7	32010.5	
96	15108.8	17182.84	16308	19745	20586	20511.4	
144	13700.4	18399.6	14306	16753	20016.7	20102.9	
192	9177.33	11841.9	9540.81	10791	9071.31	8942.31	
Degraded office paper 1-d							
	DOP 1	DOP 2	DOP 3	DOP 4	DOP lignin 1	DOP lignin 2	
0	0	0	0	0	0	0	
12	141146	140055.5	140754	139357	230704	229237	
24	69450.1	78334	79302.5	77365.5	46755.3	48031.6	
36	43930.5	43814.55	44944.3	42684.8	15714.4	15828.7	
48	28993.3	27111.35	27079.9	27142.8	6528.56	6395.85	
72	30219.5	30612.8	31405.8	29819.8	13503.6	13291.2	
96	21271.1	20607.25	20543.8	20670.7	6794.61	7336.82	
144	20118	20389.85	20551.5	20228.2	7933.85	7725.6	
192	10842.2	12660.65	11658.1	13663.2	5812.11	5070.88	

240	8392.46	8969.28	8848.57	9089.99	5013.21	4865.75	
Newsprint 30-d							
Time (hr)	NP 1	NP 2	NP 3	NP 4	NP lignin 1	NP lignin 2	NP lignin 3
0	0	0	0	0	0	0	0
12	127907	135533	145491	137372	118246.2	108001	123706.2
24	63535.3	65535.6	64545.7	58349.1	64757.29	74346.53	60184.82
36	29663.9	26156.6	26389.4	27976.7	41531.22	34278.82	39628.76
48	22570.8	23809.1	22608.6	20101.1	26853.77	21572.92	20642
72	29296.8	28519.8	27895.5	27320.1	30881.24	34189.49	32449.63
96	18633.6	18117	17583.4	17631.5	22915.24	22596.31	20542.74
144	22158.8	23011.9	23322	21950.3	22299.45	25715.19	26292.45
192	15568	15391.9	14506.2	14654.8	12797.06	9432.782	12440
240	6600.63	7798.71	6704.03	8359.83	8109.795	5914.393	8888.568
288	3488.91	4477.71	3697.3	2564.1	5990.534	4566.461	6673.185

Appendix C Relationship between optical density and CFU

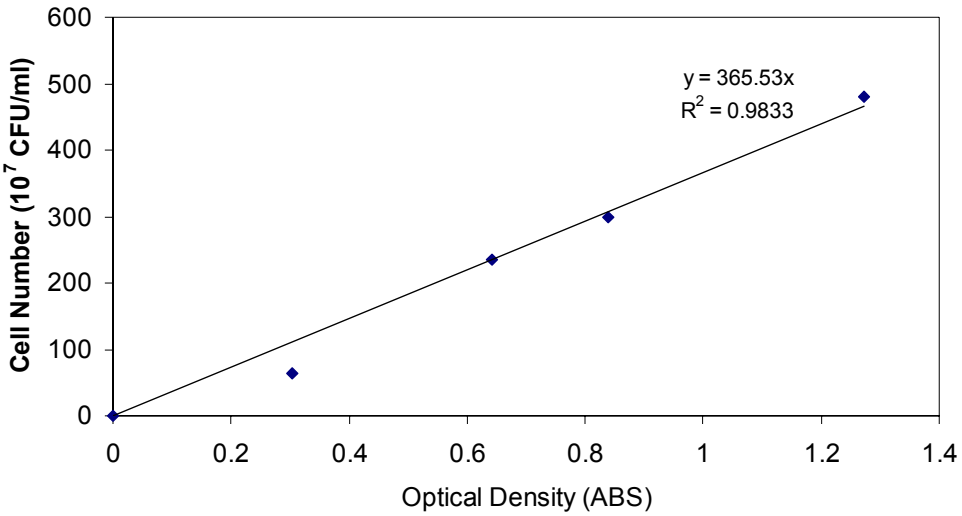


FIGURE C.1 Relationship between CFU and optical density.

CFU was measured by series dilution and plate count. Optical density was measure by spectrophotometer.

Appendix D SAS code for statistical analysis

```
options ls=90 ps=95 pageno=1;
data lignin;
proc import out=lignin datafile="D:\lignin.xls" dbms=excel2000 replace;
getname=yes; run;
proc sort data=lignin; by trt time;
title2 "comparison between each pair across all time points";
proc mixed data=lignin;
 where trt='NP' or trt='NPlignin';
 class trt time rep;
 model y=trt time trt*time/ddfm=satterth;
 repeated /type=un sub=subject;
run;

proc mixed data=lignin;
 where time=240;
 where trt='DOP' or trt='DOPlignin';
 class trt rep;
 model y=trt/ddfm=satterth;
run;
```