ABSTRACT

THE EFFECT OF COMPOSTING PROCESS PARAMETERS ON THE MINERALIZATION OF ATRAZINE

By

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Composting offers a relatively inexpensive and environmentally safe method for the potential bioremediation of pesticide-laden rinsewater. The purpose of this study was to evaluate whether co-composting of lignocellulosic substrates and pesticide would be a viable option for the disposal of the pesticide. Atrazine was chosen because it is the most extensively used pesticide in the US. Composting was carried out in 2-liter laboratory scale composters. Degradation and mineralization of atrazine were followed by the use of ^{14}C-radiolabeled atrazine. The effects of composting process parameters such as temperature, type of substrate, and inoculum on the mineralization of atrazine were studied.

Mineralization of atrazine during composting with poplar wood was investigated at three temperatures: 25°C, 37°C, and 55°C. Maximal mineralization of poplar wood carbon to CO_2 was observed at 37°C, with 10% mineralization of the poplar wood at the end of 84 days of incubation. Mineralization of atrazine was minimal in all cases.

The enhancement of degradation and mineralization of atrazine by composting with pretreated lignocellulosic materials as compared to untreated lignocellulosics was

also evaluated. Wood that was subjected to steam explosion (STEX wood) or ammonia explosion (AFEX wood), untreated wood (native), and shredded newspaper were selected as composting substrates. The results showed that there was no significant enhancement in atrazine mineralization when composted with the pretreated woods (AFEX wood and STEX wood) as compared to that observed with the native wood. Thus, pretreatment of the wood, which was hypothesized to lead to increased substrate and atrazine mineralization, was seen to have no added effect on atrazine mineralization.

Finally, the effect of an exogenous inoculum of the white-rot fungus *Phanerochaete chrysosporium* on atrazine mineralization during composting of poplar wood was investigated. The addition of an aqueous conidial suspension of *P. chrysosporium* inoculum (Strain BKM-F-1767) significantly enhanced mineralization, resulting in a 14% mineralization of atrazine in 94 days of composting compared to 1% mineralization observed in controls without the *P. chrysosporium* inoculum. Preliminary modeling and process design suggested the feasibility of co-composting atrazine and poplar wood with an inoculum of *P. chrysosporium* as a viable option for the disposal of atrazine-contaminated rinsewater.

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Chapter I: INTRODUCTION

Many pesticides commonly used in agricultural and lawn care applications pose a potential threat to public health and environmental quality. There is increasing recognition that soils at many agrichemical facilities and farms have been contaminated with high concentrations of pesticides through accidental spills or improper rinsing and discharge procedures (Long, 1989). High concentrations of ordinarily biodegradable pesticides can be persistent in soils partly because they inhibit microbial activity (Winterlin et al., 1989; Dzantor and Felsot, 1991). High concentrations of pesticides have also been found to be more mobile than low concentrations (Davidson et al., 1980). The combination of persistence and greater mobility increases the risk of surface and groundwater contamination and emphasizes the need for expeditious cleanup.

Current disposal options for these contaminated wastes include excavation and subsequent landfilling or incineration of solid wastes. Discharging of liquid wastes into drainage systems has been known to occur, with possible contamination of natural water systems (Seiber, 1991). Such options are being phased out with the passage of stricter laws aimed at curbing environmental and health problems. Disposal methods such as landfilling and incineration are expensive and do not always address the problem of contaminant detoxification. As more contaminated sites are discovered, it is becoming increasingly important to seek cleanup technologies that can be easily adapted to a variety of situations. Examples of disposal options that would fit the category of environmentally friendly technologies include in-situ remediation, landfarming (also known as land

application or land treatment) and biological treatments such as bioremediation, bioaugmentation, composting, and anaerobic digestion.

Composting, a prime example of biological treatment, is a relatively inexpensive and easily manageable alternative and has been widely used in the disposal of municipal solid wastes (Gray et al., 1973) and yard wastes (Michel et al., 1993). Composting has also been shown to be effective in the bioremediation of a variety of xenobiotics (Williams et al., 1992; Lemmon and Pylypiw, 1992; Vogtmann, 1984). The potential for bioremediation of contaminated sources such as pesticide rinsewater and contaminated soils, using composting is promising primarily because of the intensity of the microbial activity within a composting matrix. The overall transformation potential for contaminants within a composting mass is worth considering over other decontamination methods for a variety of reasons. First, elevated temperatures facilitate a higher reaction rate. Second, the opportunity for cometabolism (degradation of a recalcitrant compound or contaminant while a microorganism obtains its carbon and energy from other easily utilizable compounds) is enhanced due to the range of alternative substrates present and the high level of microbial activity. Finally, the changing physical and chemical microenvironments within a composting mass result in a diversity of microbial communities and metabolic activity, thus increasing the number and type of microorganisms to which the contaminant is exposed. Composting could be used with lignocellulosic substances as substrates, since previous studies have shown that lignocellulose degrading enzymes may also be important for the degradation of pesticides and xenobiotics (Hammel, 1992; Boominathan and Reddy, 1992). Lignocellulosic materials have also been shown to concentrate pesticides from wastewater sources with good sorption characteristics (Toller and Flaim, 1988;

Hetzel et al., 1989; Mullins et al., 1993) suggesting that co-composting of pesticides may be a disposal option. In the case of the pesticide-laden rinse water, a model lignocellulosic material such as wood could be used as the adsorption agent and the composting substrate to optimize the rates and extent of mineralization of the pesticide. The data gathered from the work could then be extended to more readily available lignocellulosics such as corn stover, corn cobs, and other agricultural byproducts.

Although research has been done in the area of pesticide degradation in soils, very little has been done in elucidating the co-composting of pesticides with lignocellulosic substrates. Research needs to be conducted in order to better understand the factors controlling the simultaneous metabolism of the lignocellulosic substrate and the various amendments in the form of pesticides or contaminated soils. The overall objective of this study was to verify whether gratuitous mineralization of pesticides was possible during the the composting of lignocellulosic substrates. This would enable us to evaluate co-composting of pesticides with various lignocellulosic materials as a viable method for disposal of the pesticide. Atrazine was chosen as the pesticide to be studied due to its widespread use in the U.S. (it accounts for about 12% of all pesticides used in this country), and due to its recalcitrance to degradation in the environment.

This dissertation is divided into seven chapters, including this first chapter (Introduction) followed by a review of relevant literature in Chapter II. Chapters III through V constitute the research papers from this dissertation. Process design and modelling are discussed in Chapter VI. Overall conclusions and future directions are presented in Chapter VII. Appendix A presents preliminary data on the effect of substrate moisture content and C/N ratio on conversion of substrate to CO_2.

Chapter II: Literature Review

Research in the fields of composting and pesticide degradation has been very diverse, encompassing a wide variety of areas such as composting substrates, parameters, and systems, fate of pesticides in the environment, and degradation of pesticides by physical, chemical, and microbiological means. Because of the diversity of research in the areas of composting and pesticides, the literature review presented here focuses on areas that have relevance to this study. This chapter presents a summary of research in the fields of composting, fate of pesticides in the environment, degradation (pure culture and in situ) of atrazine, disposal options for pesticide contaminated sources, bioremediation of pesticides using added microbial inocula, and finally, pesticide degradation during composting.

1. Composting

1.1 Composting Process

Composting is one of the many paths in nature which contribute to the closure of the carbon cycle by recycling carbon that has been used in the synthesis of various organic compounds. Composting involves the biodegradation of organic compounds by a mixed microbial consortia to carbon dioxide and water and leading to the formation of a stable humus-like product, which has been used as a soil conditioner. As a biological process it is subject to the constraints of all biological processes, namely, limitations imposed by microbial population and genetic traits, and process parameters such as temperature, moisture, and nutrients.

Composting is a method of solid waste management where the organic component of the solid waste stream is biologically decomposed to a state in which it can be handled, stored, and/or put to some end use. A variety of composting operations are in use at present that include windrow composting (passive aeration and forced aeration), and a host of in vessel systems that include rotary drums and agitated bins (Haug, 1993). A brief description of the general succession of events that occur during a common composting process (windrow composting), is presented in the next few paragraphs. A conceptual diagram of the processes that occur during composting is shown in Figure 2.1.

The composting process begins with preparing the material for composting by adjusting moisture content and/or addition of a nitrogen source. Depending on the composting material this initial step is followed by the addition of amendments such as sawdust, manure, and yard wastes, and/or bulking agents such as wood chips, to make the composting material more amenable to handling and to provide structural support and maintain air spaces in the compost matrix. The conditioned material is then placed into piles or windrows. Microbial activity in the presence of oxygen and moisture generates heat during the degradation of the organic fraction of the composting material. Temperature is a good process indicator, since the release of heat is an outcome of metabolic activity. As easily degradable compounds get utilized by the microbial species, temperature rapidly increases in the compost matrix. These thermophilic temperatures can be maintained for several weeks as seen in Figure 2.2 which represents general temperature trends that occur during windrow composting. With a decline in the amount of easily degradable substrates, microbial metabolism switches to the hydrolysis and assimilation of the polymeric materials in the compost matrix which is a relatively slower

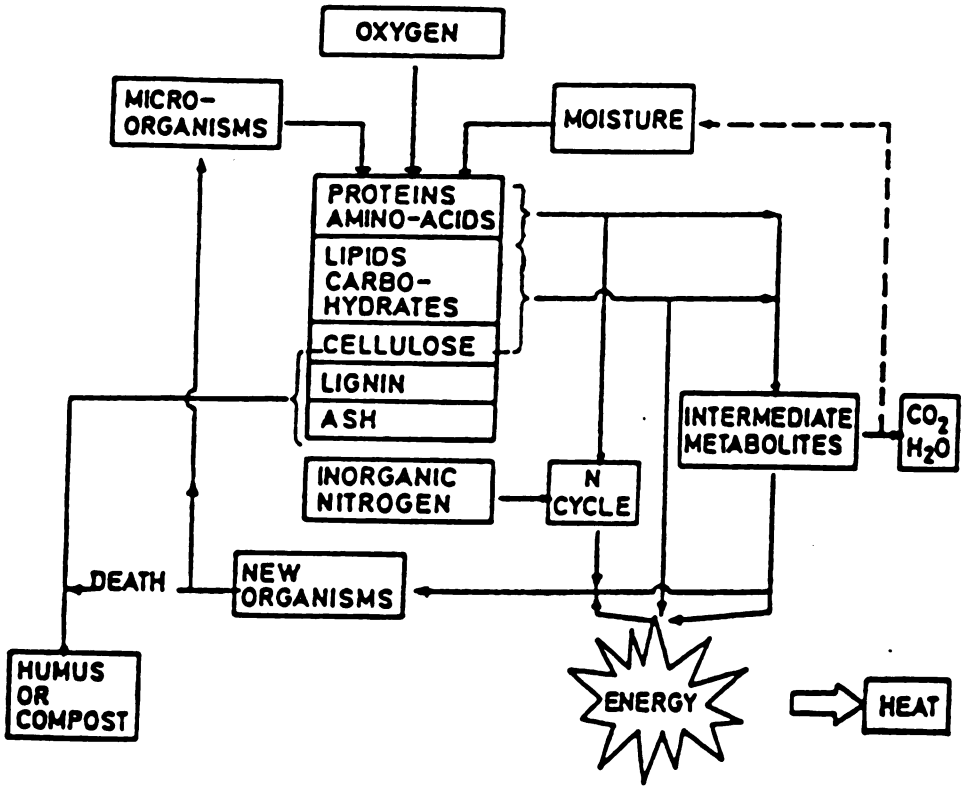


Figure 2.1: Conceptual composting process. From Gray et al. (1971a).

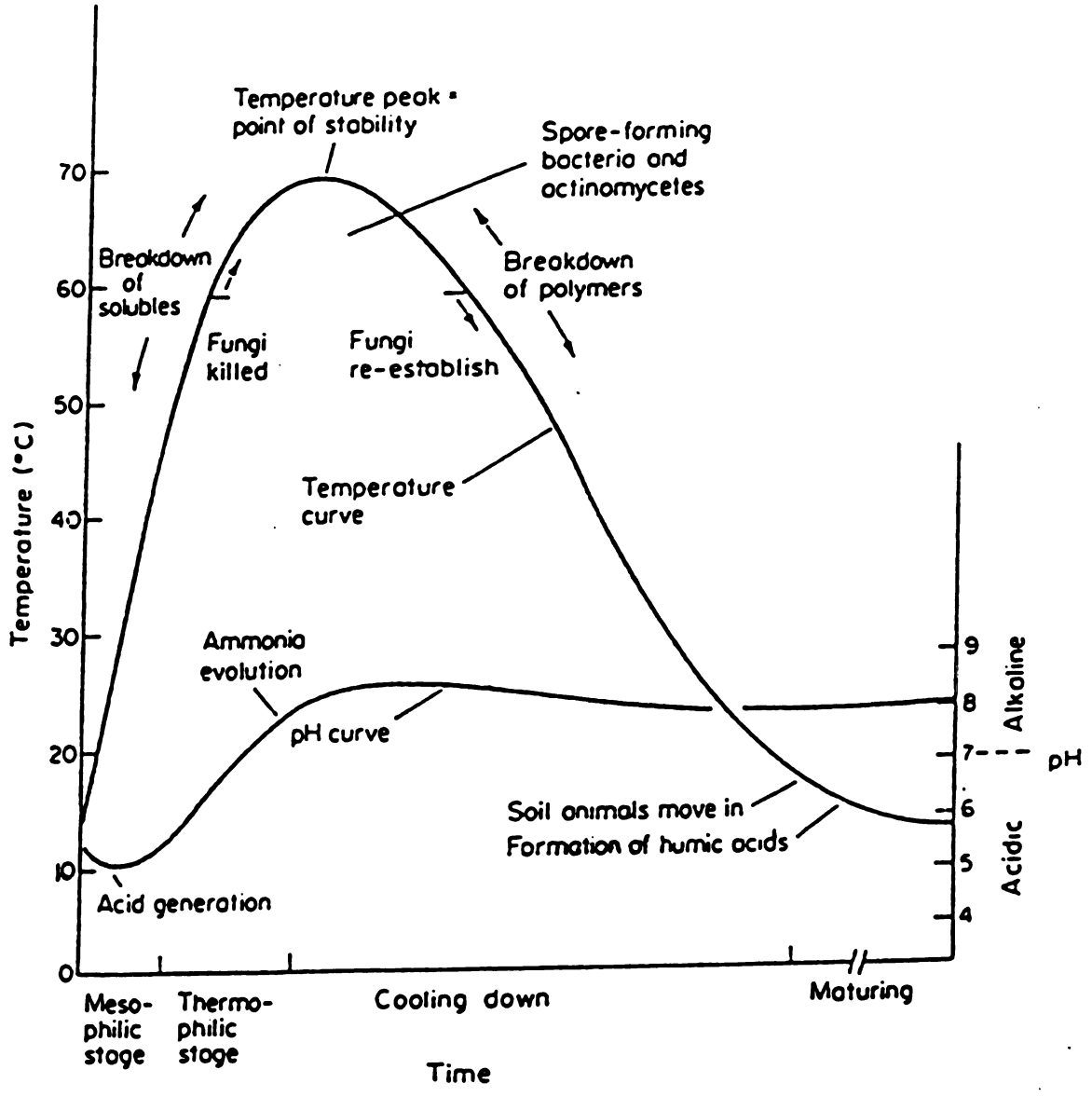


Figure 2.2: Typical temperature and pH patterns in windrow composting. From Biddlestone and Gray (1985).

process. This results in a decrease in heat generation, and temperatures gradually drop till the compost matrix reaches ambient temperatures. This characteristic temperature pattern over time reflects changes in the rate and type of decomposition taking place as composting proceeds.

The final stage of composting, a comparatively slow process compared to the previous stages, involves maturing of the composting material into a stable product. This takes place at ambient temperatures with the action of predominantly mesophilic organisms giving rise to humus and humic acids via polymerization and condensation reactions (Gray et al., 1971a). Humic acids result from condensation of plant lignin residues with bacterial protein and can also be produced by microorganisms by synthesizing carbohydrates to polycyclic compounds in the presence of nitrogen.

These processes that occur during composting are brought about by the activities of microbial communities, each of which is suited to an environment of relatively limited duration. The main classes of microorganisms encountered are bacteria and fungi, with bacteria being responsible for the initial breakdown of the organic material and for a large part of the initial heat released into the composting mass (Finstein and Morris, 1975). The initial metabolism of the easily degradable organic fraction by bacteria leads to the subsequent colonization of the compost by actinomycetes and fungi which can utilize a relatively wider array of substrates. A general drop in microbial activity is seen with the increase in temperature, with little or no activity at the peak temperatures reached in the windrow (Strom, 1985; McKinley and Vestal, 1985; McKinley and Vestal, 1984). This leads to a drop in the temperatures with recolonization of the compost by actinomycetes and fungi (Gray et al., 1971a). This period of decline of temperature to the ambient

temperature is referred to as the curing or maturing of the compost and is accompanied by a slower rate of composting and lower oxygen consumption rates. This general description of windrow composting is generally valid for most materials undergoing large-scale composting (Gray et al., 1971a,b).

1.2 Composting applications

Although a major emphasis of composting applications has been for the disposal of municipal and domestic solid wastes (Haug, 1993), composting has also been proposed as the answer to the disposal of other types of wastes. Vallini et. al. (1984) found composting to be a viable alternative for the disposal of food factory, fruit, vegetable, cork and tannery wastes. They found that tannery wastes, a highly polluting and biologically toxic substance, could be transformed quickly and inexpensively by composting, into an organic fertilizer for crops. Lopez-Real (1984) highlighted the use of high-temperature composting as a resource recovery system for agro-industrial wastes, while Biddlestone and Gray (1991) suggested composting as a source for the production of a peat alternative. The composting of a variety of substrates including tree bark, sewage sludge, municipal refuse, and newsprint has also been investigated (Ashbolt and Line, 1982; Atchley and Clark, 1979; Campbell et al., 1990a,b; DeNobili and Petrussi, 1988; Ferrari, 1987; Haug, 1979; Jeris and Regan, 1973a,b,c). Composting has also been studied as an alternate means of disposal for xenobiotics and industrial wastes (Winterlin et al., 1986; Vogtmann et al., 1984; Fogarty and Tuovinen, 1991).

1.3 Factors influencing the composting process

1.3.1 Temperature

Temperature plays an important rolein the composting process as described in section 1.1. Composting essentially takes place within two ranges of temperature (see Figure 1) referred to as mesophilic (10-45°C) and thermophilic (>45°C) though the cut off between the two ranges is ill-defined. Mesophilic temperatures allow effective composting, as observed by McKinley and Vestal (1985, 1984) who reported the greatest microbial activity in compost samples taken from lower temperature areas (35°C-45°C) of sewage sludge windrow composts. Similar findings were reported by Jeris and Regan (1973a) who investigated the bench-scale composting of newsprint and observed the highest CO_2 production rate at 48°C. They also reported that the composting of stabilized municipal refuse was found to have an optimum at 40°C in shake flask experiments. The optimum temperature during the lab-scale composting of ground garbage was found to be 45°C (Snell, 1957), while that for the solid state fermentation of straw by *Chaetomium cellulolyticum* was found to be 37°C (Abdullah et al., 1985), reflective of the lower growth temperatures for fungi. Hogan et al. (1989a) evaluated the composting of sludge amended with aliphatic and polyaromatic hydrocarbons at two temperatures (35°C and 50°C). They noted that volatile solids and hydrocarbon losses were greater at the lower temperature, keeping with the notion that higher microbial activity is generally observed at mesophilic temperatures.

Most commercial operations, however, maintain thermophilic conditions since pathogens, weed seeds, and fly larvae are destroyed at those temperatures (Composting Handbook, 1992). Microbial metabolism during composting releases large amounts of

energy as heat, resulting in an increase in the temperature of the compost due to the self-insulating qualities of the composting material (Composting Handbook, 1992). Heat accumulation can push temperatures well above 60°C which affects the compost microorganisms, leading to a slowing of the process. Temperatures can continue to rise above 70°C at which point most of the microorganisms either die or become dormant. At this stage the compost process effectively stops and does not resume until the microbial population recovers after a drop in temperature.

Researchers have also shown composting to be efficient at thermophilic temperatures. Schulze (1962) observed that temperature was directly related to microbial oxygen uptake rate between 30°C and 70°C during the small-scale composting of garbage. Suler and Finstein (1977) observed that optimum composting was achieved at 55 to 60°C during the lab-scale composting of table scrap. Strom (1985) reported that bacterial species diversity decreased markedly during the lab-scale composting of table scraps and newspaper above a temperature of 60°C and concluded that the maximum desirable temperature for composting was thus 60°C. Cathcart et al. (1986) concluded that the optimum temperature for composting unshredded and shredded crab scrap was 63°C and 56°C respectively. Attempts have also been successfully made to control the temperature at a desired set-point for optimal composting by the control of aeration to the compost matrix (Hogan et al., 1989a,b; Ryoo et al., 1991; Finstein et al., 1992).

To summarize, increase in temperature leads to an increase in the rates of degradation of the organics in the compost materials, thus increasing the rate of composting. On the other hand, the outcome of excessive increase in compost temperature is the decrease in the microbial activity in the compost, resulting in a decrease in the rate

of composting. Depending on the substrate and the composting system being used, an optimum temperature exists, at which a balance between the two opposite effects of temperature can be achieved.

1.3.2 Moisture

Since composting is a microbially mediated process, moisture plays a critical role in the composting process. Water provides the medium for chemical reactions, transportation of nutrients and enzymes, and allows for microbial movement. Desirable moisture content is linked to particle size and porosity of the composting material. In theory, optimal microbial activity is achieved at saturated conditions, with cessation of activity below a 15% moisture content. However, aeration is adversely affected at saturated conditions, possibly leading to anaerobic conditions. Thus, in practice, a range of 50-90% moisture, depending on the composting substrate, is recommended (Biddlestone and Gray, 1985). Jeris and Regan (1973b) investigated the composting of refuse containing 60-70% paper and noted that the highest oxygen consumption rate was at a moisture content of 67%. Suler and Finstein (1977) reported 60% moisture to be optimum for the lab-scale composting of table scrap. The optimum moisture content was found to lie in a range of 52 to 58% during the lab-scale composting of ground garbage (Snell, 1957). The optimum moisture content for the lab-scale solid state fermentation of straw by *Chaetomium cellulolyticum* was found to be 80% (Abdullah et al., 1985). Cathcart et al. (1986) concluded that the optimum moisture content for composting unshredded and shredded crab scrap was 67% and 55% respectively and attributed the higher optimum moisture content for unshredded scrap to larger particle size and increased porosity.

1.3.3 Aeration and Oxygen supply

Aeration serves to provide oxygen to the composting material, as well as removal of heat, water vapor, and gases that are generated during composting. Limiting the supply of oxygen slows the compost process and creates anaerobic conditions in the compost. Suler and Finstein (1977) reported that higher CO_2 evolution (indicating higher mineralization of organic carbon) was observed when the exhaust air from lab-scale composters had oxygen concentrations greater than 10%. They observed that air flow rates that left 2% O_2 in the exhaust resulted in lower CO_2 generation, emphasizing the importance of oxygen supply. This was also shown by Kaneko and Fujita (1992) who conducted lab-scale composting of newsprint and dog food at 50°C, and concluded that the efficiency of composting could be ensured by controlling the oxygen concentration in the exhaust gas to about 10%. McKinley and Vestal (1985b) observed significant improvements in the rates of microbial metabolism and growth when aeration was used to keep the temperature below 58°C during the small-scale composting of municipal sewage sludge, compared with piles composted simultaneously at higher temperatures (60 to 84°C). This highlights the interdependence of temperature, moisture content and aeration in obtaining optimal composting conditions as presented in Figure 2.3.

1.3.4 Other parameters

The composting process is also affected by other factors such as C/N ratio, pH, and particle size of the composting substrate.

Carbon and nitrogen are the primary nutrients required by microorganisms. Microorganisms use carbon for energy and growth while nitrogen is essential for protein synthesis. Since microorganisms contain approximately 50% carbon and 5% nitrogen on a

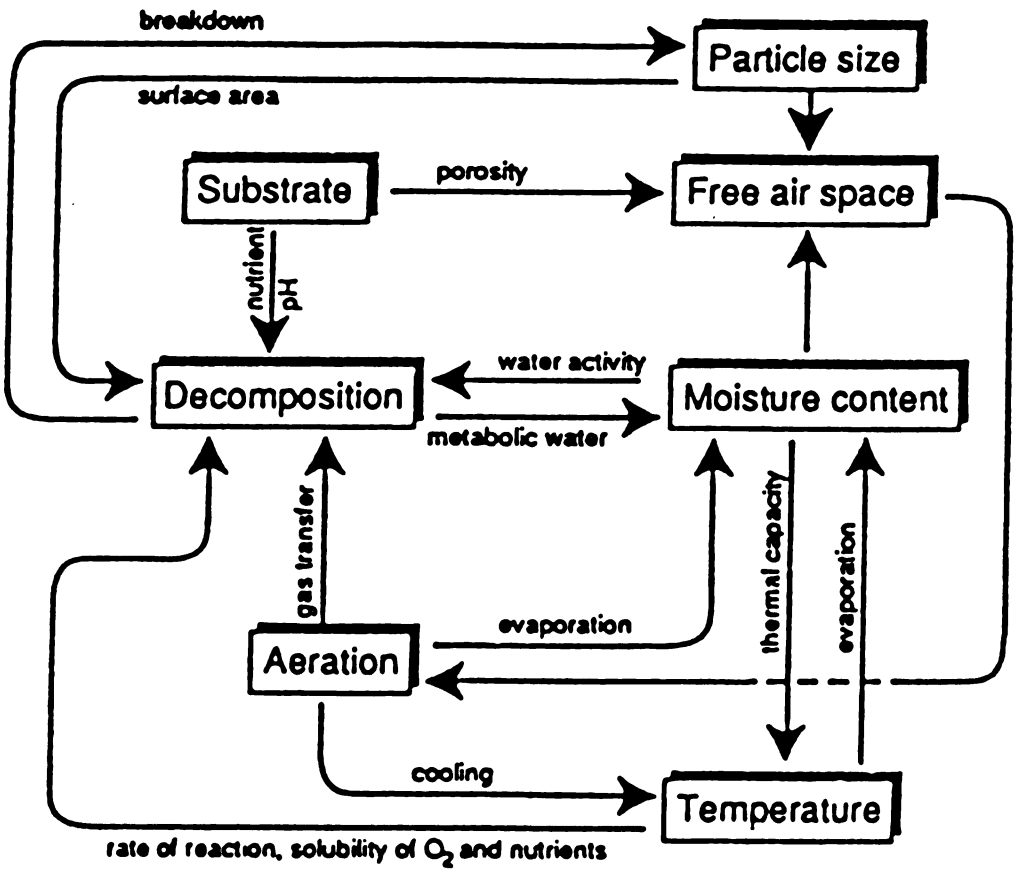


Figure 2.3: Interdependence of composting process parameters. From Campbell et al. (1990b).

dry weight basis, and approximately 20-40% of the carbon present intially in the compost material is converted to microbial biomass (Gray et al., 1971), the requirement of nitrogen in the feed is 2-4 parts/100 parts of initial carbon. Thus, raw materials with a C/N ratio of about 25:1 to 50:1 are ideal for active composting although higher ratios are also acceptable depending on the amount of available carbon (Biddlestone and Gray, 1985; Kayhanian and Tchobanoglous, 1992).

Due to the variety of microorganisms involved, the composting process is relatively insensitive to pH, especially within the range of values generally seen with composting materials. One instance where pH is a factor is in the case of materials with a high nitrogen content, where a process pH of >8.5 can lead to the formation of ammonia, creating odor problems (Composting Handbook, 1992). This can be easily controlled by adjusting the C/N ratio of the feed above 25:1.

Since microbial decomposition occurs on particle surfaces, degradability can be improved by reducing the particle size (which increases the available surface area), as long as porosity is not affected (Composting Handbook, 1992).

2. Pesticides

Pesticide usage in the U.S. has been relatively stable at about 1.1 billion pounds of active ingredient during recent years (Aspelin et al., 1991). An estimated 4-5 billion dollars worth of agricultural pesticides, corresponding to about two thirds of the total pesticide sales, are sold in the United States every year. This includes about 21,000 pesticides registered under the Federal Pesticide Law. Failure to use proper procedures at pesticide mixing and handling sites and improper disposal of pesticide laden rinsewater can result in the contamination of soil, surface water and ground water (Myrik, 1990, Norwood, 1990,

Toller and Flaim, 1988). One of the most important issues facing the agrichemical industry is the cleanup and prevention of site contamination at retail dealerships. Atrazine, alachlor, cyanazine, and metolachlor were the pesticides most frequently found in contaminated soil sites (Buzicky et al., 1992), a finding directly related to the amount of use of these pesticides (Table 2.1). A report stated that the ground water of more than half the states in the U.S. contained agricultural pesticides (Chemistry and Engineering News, 68:26-40, 1990). The findings from another report (EPA, 1988) showed the presence of 74 different pesticides in the ground waters of 38 states, more than half of which originated from agricultural uses. These reports highlight the seriousness of the extent of contamination, due to pesticide usage, and emphasize the urgent need to find cost effective solutions for the disposal or remediation of contaminated sources. However, many of the current treatment options are very expensive as observed by Myrick (1992), who estimated a cleanup cost of between three to five million dollars for just a single dealership site. He also foresaw a potential savings in the billions of dollars to the agrichemical industry contingent on the development of inexpensive site remediation technologies for contaminated dealership sites.

2.1 Atrazine

The s-triazine group of herbicides which include atrazine, simazine, and cyanizine (Figure 2.4, Table 2.2) are widely used in agriculture in the U.S. for the control of annual grasses and broad leaf weeds in corn (Cook, 1987; Aspelin et al., 1991). In addition atrazine is used extensively as a preemergent herbicide for other crops including sorghum, sugar cane, macadamia nut, and pineapple, as well as for weed control on rangeland and along railroads and highways (Herbicide Handbook, 1979). Atrazine alone accounts for

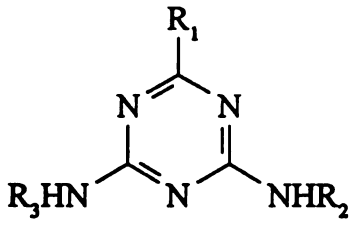
Table 2.1: Annual Usage Estimates of Pesticides in the U.S. (Aspelin et al., 1991)

Pesticide	Usage in Million Pounds Active Ingredient
Atrazine	70-90
Alachlor	60-75
2,4-D	40-65
Metolachlor	40-55
1,3-D	35-45
Trifluralin	30-40
Cyanazine	20-30
Carbaryl	10-15
Chlorpyrifos	8-16
Maneb/Mancozeb	8-12
Methyl Parathion	8-12

Table 2.2: s-Triazine herbicides and transformation products[a]

Substituent			Chemical Formula	Common Name	Abbreviation[b]
R_1	R_2	R_3			
Cl	C_2H_5	C_3H_7i	2-Chloro-4-ethylamino-6-isopropylamino-s-triazine	Atrazine	CIET
Cl	H	C_3H_7i	2-Chloro-4-amino-6-isopropylamino-s-triazine	Deethylatrazine	CIAT
Cl	H	C_2H_5	2-Chloro-4-ethylamino-6-amino-s-triazine	Deisopropyl atrazine	CEAT
Cl	H	H	Chloro-diamino-s-triazine	Dealkylatrazine	CAAT
Cl	C_2H_5	C_2H_5	2-Chloro-4,6-bis(ethylamino)-s-triazine	Simazine	CEET
Cl	C_3H_7i	C_3H_7i	2-Chloro-4,6-bis(isopropylamino)-s-triazine	Propazine	CIIT
Cl	C_2H_5	$C_3H_6(CN)$	2-[(4-Chloro-6-Ethylamino-s triazine-2-yl)amino]-2-methylpropionitrile	Cyanazine	
NH_2	H	H	2,4,6-Triamino-s-triazine	Melamine	AAAT
OH	C_2H_5	C_3H_7i	2-Hydroxy-4-ethylamino-6-isopropylamino s-triazine	Hydroxyatrazine	OIET
OH	H	C_3H_7i	2-Hydroxy-4-amino-6-isopropylamino s-triazine	Deethyl hydroxyatrazine	OIAT
OH	C_2H_5	H	2-Hydroxy-4-ethylamino-6-amino s-triazine	Deisopropyl hydroxyatrazine	OEAT
OH	H	H	2-Hydroxy-4,6-diamino s-triazine	Ammeline	OAAT

a - Refer to Figure 2.4(a) for general structure of *s*-triazines
b - From the system developed by Cook (1987)



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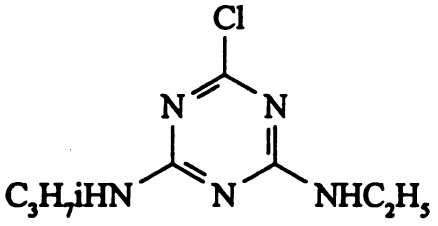
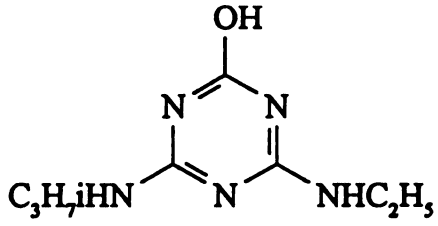
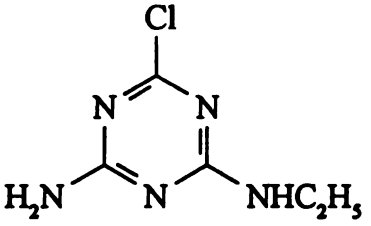


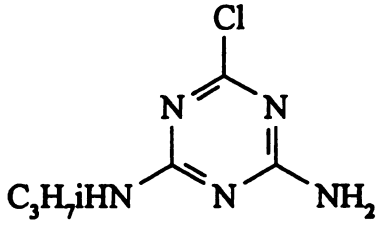
Atrazine



Hydroxyatrazine



Deisopropylatrazine



Deethylatrazine

b)

Figure 2.4: a) General structure of *s*-triazines (See Table 2.2 for substituents)
b) Structure of atrazine and common metabolites

about 12% of all the pesticides used in the U.S. and is most heavily used in the midwest. Over 36 million kilograms of atrazine were applied nationwide in 1990 (Periera and Rostad, 1990). Atrazine and its metabolites, deethylatrazine and deisopropylatrazine (Figure 2.4b) are the most frequently found pesticides in surface waters of the midwestern U.S. (Thurman et al., 1991). Atrazine has been detected in lakes and streams at levels ranging from 0.1 to 30 μg/L with peak concentrations up to 1000 μg/L known to occur in surface runoff from agricultural fields adjacent to bodies of water during times of application (Day, 1991). These concentrations generally exceed the maximum contaminant level of 3 μg/L that took effect in 1992 (EPA, 1991).

2.2. Fate of pesticides in the environment

Degradation and sorption are two of the most important processes governing the fate of pesticides in the environment. Processes involved in the degradation of pesticides can be classified under three main categories: physical, chemical, and microbiological. The two primary physical processes involved in the degradation are light and heat. Photolysis of pesticide residues is extremely significant on vegetation, on the soil surface, and in water (Coats, 1991). Thermal decomposition often occurs in conjunction with the photodegradative reactions. Solar radiation is therefore responsible for the degradation by both, photolysis and thermal decomposition.

Chemical degradation occurs as a result of the various reactive agents in the pesticide formulations and in the environment. Water is responsible for considerable breakdown of pesticides in solution, especially in conjunction with pH for pH sensitive compounds. In most environments, oxidative reactions involving oxygen, ozone and

peroxides are the most frequent degradative pathways observed (Hapeman-Somich, 1992).

Biological agents are also significant in the degradation of pesticides. Microorganisms are the most important group of degraders based on their prevalence in the environment. The major strategies exhibited by the microbes include catabolism, co-metabolism, and extracellular enzymatic activity in the presence or absence of microbes (Coats, 1991).

The influence of sorption on the biodegradation of organic contaminants has been recognized as an important issue in environmental science (Alexander, 1991). Factors cited as reducing the availability of pollutants for biodegradation included sorption to soils, presence in a physically inaccessible state, and binding of the pesticides in a manner as to prevent their transformation by microbial activity (Alexander, 1991). Sorption has been generally considered to hinder or limit the rate and extent of pesticide mineralization, as indicated by Ogram et al. (1985) and Weissenfels et al. (1992). But Guerin and Boyd (1992) showed that the availability of sorbed naphthalene was different for two different bacterial species, and that generalizations regarding the bioavailability of sorbed organic contaminants and pesticides were inappropriate.

Similar to sorption, the contact time between pesticides and soils, referred to as pesticide aging is another important factor influencing the bioavailability of pesticides. Steinberg et al. (1987) compared the biodegradation of residual ethylene dibromide in field weathered soils to those of freshly added ethylene dibromide. Dramatic differences were observed showing aged ethylene dibromide to be persistent, whereas freshly added radiolabeled ethylene dibromide was readily degraded within the same soil sample.

2.3. Degradation of atrazine

Atrazine, along with the others in the s-triazine group, are relatively persistent in the environment, with the most heavily substituted and chlorinated s-triazine analogs being the least biodegradable. However, both bacteria and fungi have been shown to mineralize atrazine to varying degrees (Cook, 1987), though atrazine uptake and metabolism was very limited in algae (Jones et al., 1984; Butler et al., 1975). Atrazine can be degraded by either biotic or abiotic processes. (Mandelbaum et al., 1993b). N-dealkylation, dechlorination and hydroxylation at the 2-position, deamination, and ring cleavage are the major degradative processes for atrazine. Biotic degradation of atrazine generally results in the production of desethylatrazine or desisopropylatrazine (Levanon, 1993) with the mineralization of the alkyl-amino side chains mainly due to fungal activity. Research has shown deethylatrazine to be the more stable and dominant initial biotic degradation product. The abiotic degradation of atrazine results in hydrolysis with the initial formation of hydroxyatrazine. Deethylatrazine is almost as phytotoxic as atrazine while deisopropylatrazine is five times less phytotoxic. Dealkylatrazine (2-chloro-4,6-diamino-s-triazine) and hydroxyatrazine are non-phytotoxic (Winkelmann and Klaine, 1991).

2.3.1. Abiotic Degradation

As mentioned earlier in section 2.2, abiotic degradation of pesticides by physical or chemical processes has been shown to contribute to the overall degradation of the pesticide. There have been quite a few studies on the effect of abiotic factors on the degradation and possible mineralization of atrazine. Hapeman-Somich et al. (1992) reported that the aqueous ozonation of atrazine at a concentration of 0.153 μM (33 ppm) gave rise to a variety of products including CIAT, CDIT, CEAT, and CAAT. They also

found that the s-triazine ring remained intact and that the chorine atom was not removed. Similar results were obtained by Adams and Randke (1992) with the exception of recovery of hydroxy metabolites of atrazine. A study by Hance (1979) on the effect of soil pH on the degradation of atrazine showed that degradation was relatively insensitive to soil pH ranging from 5.1 to 8.2. On the other hand, Best and Weber (1974) reported that atrazine degradation in a soil at pH 5.5 was greater than that observed in the same soil adjusted to pH 7.5. Moyer and Blackshaw (1993) found that atrazine dissipation was related to the amount of rainfall received (and thus the soil moisture content), with greater amounts of rainfall leading to a correspondingly greater dissipation of atrazine. A modified Fenton system (Pratap and Lemley, 1994) consisting of electrochemical generation of iron in the presence of hydrogen peroxide was shown to be effective in degrading 90% of an aqueous solution of atrazine in about 2 hours. Another process that was studied was the vacuum-ultraviolet photolysis of atrazine in water (Gonzalez et al., 1994). Up to 50% of the initial atrazine was found to be degraded to cyanuric acid though the extent of mineralization to CO_2 was minimal.

Ro et al. (1995) found that sodium azide could chemically transform atrazine to 3-ethylamino, 5-isopropylamino-s-triazyl azide and 3-ethylamino, 5-isopropylamino-s-triazinone. A complete dissipation of 10 mg/L of atrazine was observed with a 1% sodium azide solution, within 21 days of anaerobic incubation. Koskinen et al. (1994) observed a 50% degradation of analytical grade and formulated atrazine in 330 and 2772 minutes respectively during ultrasonication of aqueous solutions of the pesticide. No mineralization of atrazine was observed. Widmer et al. (1993) found no significant loss in atrazine concentration to occur during storage in well water and deionized water for 19 weeks.

Almost all the systems described above used very dilute aqueous solutions of atrazine (up to 33 ppm) and were not shown to mineralize atrazine. These methods showed increased rates of degradation of atrazine to its metabolites but suffered the drawback of being unable to effect atrazine mineralization. Thus the use of such systems in atrazine disposal strategies would necessitate additional steps to further eliminate the metabolites created.

2.3.2. Pure Culture Degradation

Donnelly et al. (1993) studied the degradation of 2,4-D and atrazine by nine different fungal species. The pesticides were used at two concentrations, 1 and 4 mM, corresponding to 215 and 860 ppm of atrazine, and at three nitrogen levels: 0, 1, and 10 mM. They found that there was no significant mineralization of the ^{14}C-ring labeled atrazine at the end of 8 weeks, though the cultures grew at both atrazine concentrations. This was in agreement with the findings of Kaufman and Blake (1970) who found no mineralization of ^{14}C-ring labeled atrazine by soil fungi, though mineralization by N-dealkylation was observed when ^{14}C-chain labeled atrazine was used. In contrast, enrichment cultures containing ^{14}C-ring labeled atrazine at a concentration of 100 ppm were shown to mineralize 80% or more of atrazine to $^{14}CO_2$ in 3 days (Mandelbaum et al., 1993a). This was attributed to the use of citrate and sucrose as mixed carbon sources and atrazine as the sole nitrogen source. The differences in the findings of these groups could be attributed to the fact that atrazine degradation and mineralization might be mediated by a mixed microbial consortia. This hypothesis is supported by the finding of Mandelbaum et al. (1993a) who noticed that more than 200 pure cultures isolated from the enrichment

cultures failed to utilize atrazine, whereas mixing these pure cultures restored atrazine mineralization.

Behki and Khan (1986) isolated three species of *Pseudomonas* capable of utilizing atrazine as a sole source of carbon, from soil with a long history of atrazine application. Atrazine was metabolized via N-dealkylation with a preferential formation of deisopropylatrazine over deethylatrazine. These researchers also isolated a *Rhodococcus* strain (B-30) which rapidly degraded atrazine to its mono and di dealkylated analogs in 72 hours (Behki and Khan, 1994). The authors indicated that the presence of both alkyl groups and the presence of chlorine at the 2-position was necessary for the N-dealkylation of either alkyl group by the bacterial strain. This meant that the metabolites of atrazine could not be degraded further by the bacteria.

Thus, atrazine metabolism has been observed to mainly occur by N-dealkylation and hydrolysis, with minimal degradation by ring cleavage, although recent efforts at isolating organisms capable of extensive mineralization of atrazine by ring cleavage have been successful. Radosevich et al. (1995) isolated a bacterial species which mineralized 40% of the initial ring labeled atrazine (22 ppm) in about 100 hours, while Yanze-Kontchou and Gschwind (1994) reported the isolation of a *Pseudomonas* strain (DSM 93-99) which was capable of mineralizing 50% of a 30 ppm atrazine solution in 50 days. Atrazine metabolites detected at the end of the 50 days were cyanuric acid and hydroxyatrazine. Mandelbaum et al. (1995) reported isolating a *Pseudomonas* species that could mineralize 80% of a 100 ppm atrazine solution to $^{14}CO_2$ in 150 hours. This bacterium was also shown to degrade atrazine at concentrations of 1000 ppm in agar plates by the appearance of clearing zones on indicator agar plates.

A summary of the research on the degradation of atrazine by pure cultures of organisms is presented in Table 2.3.

2.3.3. In situ Degradation

Numerous studies have been conducted to date on the degradation and mineralization of atrazine by microorganisms in situ in soils and other habitats (Erickson and Lee, 1989; Cook 1987).

Kolpin and Kalkhoff (1993) observed a substantial decrease in atrazine concentration in a 11.2 km stretch of water in a creek in Iowa. They found that the concentrations of two biotic atrazine degradation products (CIAT and CEAT) were constant or decreasing downstream, suggesting an abiotic degradation process, possibly photolytic.

Assaf and Turco (1994) found that atrazine degradation in soils amended with carbon as mannitol, and with nitrogen as urea at levels of 10, 30, 50, or 80 mg/kg was similar to degradation in unamended soils. They observed that 39% of applied atrazine was mineralized after 326 days regardless of the initial carbon or nitrogen treatment. The major metabolite recovered was hydroxyatrazine with lesser quantities of the dealkylated metabolites also detected. Hance (1973) showed that the addition of inorganic nutrients and straw to soils doubled the rate of degradation of atrazine and decreased the half life of atrazine by 50%, though the effect of amendments on atrazine mineralization was not investigated.

Winkelmann and Klaine (1991) reported that 59% of the ring labeled dealkylatrazine and 21% of the ring labeled hydroxyatrazine were mineralized by soil microcosms after 180 days of incubation. They also reported an exponential decrease in

Table 2.3: Summary of atrazine degradation research in pure cultures

Researcher(s)	Amount of Atrazine (ppm)	Microorganism(s)	Findings[a]
Mandelbaum et al. (1993a)	100	Mixed bacterial culture	80% in 3 days
Mandelbaum et al. (1995)	100	*Pseudomonas* sp.	80% in 150 hours
Radosevich et al. (1995)	22	Bacterial sp.	40% in 100 hours
Yanze-Kontchou and Gschwind ('94)	30	*Pseudomonas* sp.	50% in 50 days
Donnelly et al. (1993)	215, 860	Nine different fungi	(b)
Kaufman and Blake (1970)	5	Twelve soil fungi	(b)
Behki and Khan (1986)	50	*Pseudomonas* sp.	(c)
Behki and Khan (1994)	15	*Rhodococcus* sp.	(c)
Mougin et al. (1994)	0.43	*P. chrysosporium*	(b), (c)
Selim and Wang (1994)	0.2	Granular activated carbon bed	99% decrease in atrazine concentration

a - Percentages refer to amount of mineralization of ^{14}C-ring-labeled atrazine to $^{14}CO_2$
b - No mineralization by ring cleavage
c - Mineralization of atrazine by N-dealkylation only

atrazine concentration over a period of 180 days, with > 90% disappearance in the first 60 days. Bacterial mixed cultures which had been previously reported to mineralize atrazine in liquid growth medium (Mandelbaum et al., 1993a), were further used in a study to determine whether they could metabolize atrazine in soil (Mandelbaum et al., 1993b). More than 90% of both atrazine and hydroxyatrazine were degraded after 24 hours of inoculation with the bacterial cultures, though the mineralization of atrazine obtained in pure cultures was not observed in soils.

Wolf and Martin (1975) studied the degradation of ring labeled atrazine, cyanuric acid, and dealkylatrazine in soils and in pure culture. They observed an 18% mineralization of atrazine to $^{14}CO_2$ after 550 days of incubation as compared to 40% mineralization of dealkylatrazine in 192 days, and 87% mineralization of cyanuric acid in 16 days.

Ro and Chung (1995), investigating the aerobic biotransformation of atrazine in wetland sediment, found that atrazine concentration dropped from 10 mg/L to less than 10 µg/L within three weeks of incubation at room temperature. In a related study, Chung et al. (1995) observed a much slower rate of degradation of atrazine under anaerobic conditions, with atrazine (10 ppm initial concentration) dropping to below detectable levels after 38 weeks of incubation.

Solid-state fermentation of atrazine using bioreactors containing nutrient enriched peat moss resulted in an 86% disappearance of atrazine at the end of 26 weeks (Mullins et al., 1993), while the use of bioreactors containing steam exploded wood was shown to decrease solvent extractability of atrazine by 80% within 320 days (Berry et al., 1992)

On the other hand, researchers have reported atrazine to be recalcitrant to ring cleavage in various environments including alluvial-aquifer sediments (McMahon et al.,

1992), natural aquifers (Agertved et al., 1992), soils inoculated with *Phanerochaete chrysosporium* (Hickey et al., 1994), and soils (Nair and Schnoor, 1992; Dao et al., 1979; Skipper and Volk, 1972; Skipper et al., 1967; Goswami and Green, 1971).

The ultimate fate of atrazine in soils was investigated by Schiavon (1988) who found 49 to 67% of the initial ^{14}C radiolabeled atrazine in bound residues in the 0 to 6 cm level of soil columns incubated under field conditions for a year. Bidealkylated atrazine (CAAT) was found to form the highest amounts of bound residues followed by the monoalkylated metabolites and last of all by hydroxyatrazine. Khan (1991) reported that 54% of the initial ^{14}C-atrazine (25 ppm) was found in bound residues after an incubation period of a year. Analysis of the residues revealed the presence of atrazine (3.7 ppm), and its metabolites hydroxyatrazine (1.5 ppm), deethylatrazine (2.1 ppm) and deisopropylatrazine (1.1 ppm). Similar results were observed by Winkelmann and Klaine (1991) who showed that, after 180 days incubation with soil microcosms, soil bound residues of atrazine and its metabolites accounted for as much as 60% of the initial radioactivity applied (as atrazine) to the microcosms.

Blumhorst and Weber (1994) conducted experiments to investigate the relationship between chemical and microbial degradation of atrazine in soils ranging in pH from 5.3 to 8.1. They found atrazine degradation to be dominated by chemical processes at moderately acidic pH and by microbial processes at neutral pH.

The research discussed in this section has been summarized in Table 2.4.

Table 2.4: Summary of atrazine (in situ) degradation research

Researcher(s)	Amount of atrazine (ppm)	Environment	Findings
Assaf and Turco (1994)	10	Soils	39% mineralization in 326 days
Levanon (1993)	1	Soils	29% mineralization in 32 days
Wolf and Martin (1975)	2.5	Soil	18% mineralization in 550 days
Nair and Schnoor (1992)	0.37	Soil microcosms	1.5% mineralization in 100 days
Skipper and Volk (1972)	2-3	Soil	0.1% mineralization in 2 weeks
Ro and Chung (1995)	10	Wetland sediment	99% degradation in 3 weeks
Winkelmann and Klaine (1991)	2	Soil	90% degradation in 60 days
Hance (1973)	10	Soils + amendments (Nutrients/Straw)	Degradation rate doubled with amendment addition
Hickey et al. (1994)	25	Soil amended with *P. chrysosporium*	(a)
Goswami and Green (1971)	10-20	Submerged soils	(a)
Dao et al. (1979)	1.5	Soil	(a)
Agertved et al. (1992)	0.4	Natural aquifer	No degradation

(a) - No mineralization of atrazine by ring cleavage observed

2.4. Disposal Options for Pesticide Contaminated Sources

The two main options currently available on a commercial basis for the disposal of pesticide contaminated water are evaporation/degradation and filtration technologies (Seiber, 1991). Evaporation based approaches include the pouring of the pesticide laden water on a bed of soil or some other matrix and allowing for the concentrating of the pesticide on the matrix by natural evaporation. The next step would be the degradation of the concentrated pesticide by the action of sunlight, microorganisms, chemical reactions, etc. The filtration process employs a similar strategy with activated charcoal as the matrix for adsorption/concentration of the pesticide. Though these processes address the issue of pesticide removal from waste streams, they still face the problem of disposal of the pesticide laden matrices. Options for disposal include composting, microbial treatment, incineration, and encasement.

Research has been conducted with different matrices as adsorbents for pesticide laden wastes. Mullins et al., (1989) found peat moss to be an effective adsorbent for the pesticide diazinon, and observed a drop in diazinon concentration from initial values of 4000 to 32000 mg/kg to about 1 to 7 mg/kg after 18 weeks.

Removal efficiencies of up to 98% were observed for the pesticides paraquat, diquat, and amitrole, using chemically modified peat as the adsorbent (MacCarthy and Djebbar, 1986). Extractive liquid membrane technology was used to assess the feasibility of extracting pesticides from rinsewaters typical of those arising at dealership sites (Norwood, 1992). Results showed a removal of 85.4 and 92.9% of 2,4-D and atrazine respectively after 15-20 minutes of mixing time.

Dennis and Kobylinski (1983), studied the effect of adsorption of seven different pesticides by a granular activated carbon system. They observed that, at pesticide loading rates of up to 100 ppm, absorption efficiencies of 95% and greater were achieved in 21 hours by 45 lb of granular carbon. A simple activated charcoal filtration system, with a startup cost of about $1200, was investigated by Massey et al. (1992). They observed a drop in pesticide concentrations from initial values of 300 to 1000 ppm to less than 10 ppm after 120 minutes of filtration through, and adsorption by the activated charcoal. Incubation of alachlor treated charcoal with a mixed culture of microorganisms resulted in approximately a 50% loss of alachlor after 50 days.

Hunter (1992) showed supercritical extraction using CO_2 to be a viable alternative for the removal of pesticides from contaminated soils, with >95% removal of atrazine, bentazon, alachlor, and permethrin. The advantages of using CO_2 were that CO_2 is non-polluting, inexpensive, and easily recycled. The disadvantages were the high cost, high operating pressure (3000 psi), and the fact that different contaminants could require different extracting conditions.

Martinez-Inigo and Almendros (1992) found the addition of composted evergreen oak to soils significantly enhanced the sorption of atrazine as compared to composted evergreen alone.

2.5. Bioremediation of Pesticides Using Added Microbial Inocula

Dzantor and Felsot (1991) found that introducing an alachlor cometabolizing fungus into contaminated soil at 0.015% w/w caused a marginal increase in the degradation of 100 ppm of alachlor. Increasing the amount of inoculum to 0.045% w/w showed a slightly higher rate of alachlor loss (50% of the initial alachlor at the end of 55

days, as compared to 40% with 0.015% inoculum and 30% with no inoculum). They also found that amending the soils with 2% corn residue completely masked the effects of fungal inoculation.

Addition of a pentachlorophenol utilizing *Arthrobacter* to soil enhanced the removal of the pesticide (Edgehill and Finn, 1983). Similar results were observed by inoculation of contaminated soil with a pentachlorophenol degrading *Flavobacterium* species, however, several inoculations were required for substantial removal of the pesticide (Crawford and Mohn, 1985). The reason for the rapid decline of the *Flavobacterium* species was thought to be the inability to compete with the native microorganisms. Repeated applications of Pseudomonas cepacia strain AC1100 were also needed to maintain biodegradation of 2,4,5-T in soil, and the strain rapidly died in the absence of 2,4,5-T (Kilbane et al., 1983). Successful field scale bioremediation of soils contaminated with up to 9000 mg of chlorophenols per kg of dry soil by composting has been demonstrated (Valo et al., 1986).

Bioremediation has its basis in the physiology and the ecology of the microorganisms. The survival and activity of externally introduced microorganisms in any environment is an important factor in attempts to use them in field applications. Cleanup methods thus have to be designed around the capabilities of the microorganisms, which requires detailed knowledge of the biodegradation pathways and the requirements and behavior of the microorganisms.

3. Pesticide Degradation During Composting

Composting has proved to be an effective technology for the bioremediation of nitrated aromatics and nitrated triazine explosives contaminated soils (Williams et al.,

1992; Williams et al., 1990; Doyle et al., 1986). In addition, a wide variety of pesticides including atrazine, 2,4-D, diazinon, parathion, chlorpyrifos, pendimethialin and benomyl have been shown to degrade/disappear during composting (Vogtmann, 1984; Lemmon and Pylypiw, 1992). Harrad et al. (1991) found municipal yard waste composting to concentrate and increase the amounts of chlorophenols, chlorobenzenes, PCDDs, and PCDFs. Compost amendments were found to enhance the degradation of 2,4-D, MCPA, and benthiocarb in soils with complete degradation of the herbicides in 8 days as opposed to 13 days without the amendment (Duah-Yentumi and Kuwatsuka, 1982). These researchers also studied the effects of anaerobic (reductive-flooded) and aerobic (oxidative-flooded) conditions on the degradation of the same herbicides and found that compost amendments promoted the degradation of the pesticides under aerobic conditions, whereas under anaerobic conditions the degradation was very slow, with compost amendments having no effect on the process (Duah-Yentumi and Kuwatsuka, 1980).

Rose and Mercer (1964) explored the potential of composting for decomposing diazinon, parathion, DDT, and dieldrin. They found that composting lowered the diazinon concentration by 98% in 42 days, while a 50% degradation of parathion was noticed in 12 days. They found that composting had a minimal effect on DDT and dieldrin. Research by others (Wilson et al., 1983; Geunzi and Beard, 1968) showed that these recalcitrant compounds (DDT and dieldrin) could be degraded using a combination of techniques such as composting followed by anaerobic digestion.

The behavior of five pesticide residues was studied during aerobic and semi-anaerobic composting of cotton gin wastes (Winterlin et al., 1986). Propargite,

methidathion, and chlorate residues declined significantly during both composting treatments, while DEF and paraquat were stable to composting. Vogtmann et. al. (1984) demonstrated that greater than 80% degradability could be obtained on average for a variety of pesticides. However, degradation of the pesticides in both studies was based on the disappearance of the pesticides and not on complete mineralization.

Thus there is ample evidence of the ability of compost matrices to degrade a broad range of pesticides. Despite the publication of guidelines for the use of composting in the treatment of hazardous materials and industrial wastes (Becker et al., 1985; Kaplan and Kaplan, 1982; Willson et al., 1982; Muller and Korte, 1976) there is a lack of information regarding the processes involved and the ultimate fate of xenobiotics and pesticides in composting systems (Fogarty and Tuovinen, 1991).

3.1 Pesticide Degradation during Composting of Lignocellulosics

One avenue of research that has not been well investigated is the co-metabolism of pesticides during the composting of lignocellulosic substrates. Lignocellulosic materials are an ideal choice as a composting substrate because they have been shown to concentrate pesticides from wastewater sources owing to their high sorption characteristics (Mullins et al., 1993; Hetzel et al., 1989; Toller and Flaim, 1988). Furthermore, enzyme systems of certain lignocellulose degrading organisms might also gratuitously degrade xenobiotics due to their non-specificity, and structural similarity of the xenobiotics to portions of the lignocellulose substructure. The non-specificity arises from the fact that these enzyme systems are oxidative in nature (rather than hydrolytic), using free radical intermediates to accomplish the task of degrading the substrates (Reddy, 1995; Boominathan and Reddy, 1992; Hammel, 1992; Bumpus et al., 1985).

The main class of organisms exhibiting this phenomenon are the lignin degrading white rot fungi, of which *P. chrysosporium* has been the most widely studied (Boominathan and Reddy, 1992; Crawford and Crawford, 1980). These organisms possess lignin degrading enzyme systems that have been shown to degrade a wide variety of pesticides, PCB's and other xenobiotics (Reddy, 1995; Boominathan and Reddy, 1992; Hammel, 1992; Bumpus et al., 1987; Bumpus et al., 1985).

4 Research Objectives

The objectives of this study, based on the hypothesis that gratuitous mineralization of atrazine is possible during the composting with lignocellulosic substrates, are:

1. To investigate the potential for the gratuitous degradation and mineralization of atrazine during the composting of lignocellulosic substrates.

2. To study the effect of composting process parameters such as temperature, moisture, and substrate composition on the mineralization of atrazine.

3. To study the effect of the addition of an exogenous inocula of *P. chrysosporium*, a wood degrading fungus, on the co-mineralization of wood and atrazine.

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Chapter III

Effect of Temperature on the Transformation of Atrazine During the Composting of Poplar Wood and Corn Cobs

(Manuscript)

ABSTRACT

Mineralization of atrazine during the composting of poplar wood was investigated at three different temperatures: 25°C, 37°C, and 55°C. In addition, the effect of poplar wood as the composting substrate versus corn cobs as the substrate on atrazine mineralization was investigated at 55°C. Maximal mineralization of poplar wood carbon to CO_2 was observed at 37°C, with 10% mineralization of the poplar wood at the end of 84 days of incubation. Also, the extent of mineralization of corn cobs was higher at 55°C (>15%) than that of poplar wood as substrate at 55°C (<6%). Mineralization of atrazine was minimal in all cases.

INTRODUCTION

Composting is an easily manageable, environmentally safe, and relatively inexpensive alternative for the disposal of municipal solid wastes and yard wastes (Fogarty and Tuovinen, 1991; Michel et al., 1993). Lignocellulosic materials are an ideal choice as a substrate for the disposal of pesticides via composting, because lignocellulose degrading enzymes may be important for the degradation of pesticides and xenobiotics (Reddy, 1995; Boominathan and Reddy, 1992; Hammel, 1992; Fogarty and Tuovinen, 1991; Bumpus and Aust, 1987). Furthermore, they have been shown to concentrate pesticides from wastewater sources owing to their high sorption characteristics (Mullins et al., 1993; Hetzel et al., 1989).

Atrazine degradation and mineralization in soils and by microorganisms in vitro has been studied extensively at mesophilic temperatures (Erikson and Lee, 1989; Cook, 1987). Kaufman and Blake (1970) reported that no $^{14}CO_2$ was evolved from ^{14}C-ring labeled

atrazine in pure culture solutions of soil fungi maintained at 24°C. Nair and Schnoor (1992) found about 1% mineralization of atrazine in 125 days in soils incubated at 25°C. McCormick and Hiltbold (1966) investigated the microbial degradation of atrazine in soils at different temperatures. They concluded that the rate of degradation approximately doubles with each 10 degree increase from 10°C to 30°C. However, there is no information on the relative rate and extent of mineralization of atrazine when it was composted with lignocellulosic substrates at different temperatures. The rate and extent of mineralization of atrazine during composting of lignocellulosic substrates at three different temperatures, 25°C, 37°C, and 55°C are presented in this paper.

MATERIALS AND METHODS

Pesticides

[U-ring-^{14}C]Atrazine (specific activity: 7.8 mCi/mmol; purity>98%) was obtained from Sigma Chemicals, St. Louis, MO. AAtrex 4L, a commercially available sprayable atrazine emulsion (50% active ingredient), was obtained from Ciba-Geigy (Greensboro, NC). Analytical grade atrazine was obtained from Chem Service (West Chester, PA).

Compost Substrates

The poplar wood used in this study was provided by the NSF Center for Microbial Ecology at Michigan State University, and is a hybrid between *Populus nigra* and *Populus deltoides* and has been designated *Populus x euramericana* cv. Eugenei. The wood was ground in a Wiley mill through a #10 screen (1.7 mm mesh size) and adjusted to 70% moisture (g/g wet wt.) by adding distilled water. Corn cobs obtained from a local store (Soldan's Pet Supplies, Lansing, MI) were also adjusted to 70% moisture and used as a

substrate in another experiment. Each substrate was loaded into duplicate composters to give 100 g dry substrate per composter. The composters were amended with [U-ring-^{14}C]Atrazine (5.6 μC per composter) and AAtrex (500 mg atrazine/kg dry substrate). The inoculum (10% w/w) used for composting was obtained from 10-week-old wood compost piles operated by a large scale composting facility (Hollandia Gardens, Holland, MI).

Composting System

Composting was carried out in a laboratory scale composting system recently described by Michel et al. (1993). The effect of temperature on atrazine mineralization was studied under laboratory conditions by running the composters at three different temperatures: 25°C, 37°C, and 55°C. In the last case, the composters were started out at room temperature in a temperature programmable incubator, the temperature ramped to rise at a rate of 5°C a day up to a temperature of 55°C, and then held constant at 55°C for the duration of the experiment to simulate temperature patterns generally observed in windrow composting using yard trimmings as the substrate (Michel et al., 1993). The second set of composters were placed in a temperature controlled room maintained at 37°C, while the third set of composters were incubated at ambient temperature (~25°C).

Procedures for measuring CO_2 Evolution and Fractionation of the Compost.

Total CO_2 as well as the amount of $^{14}CO_2$ evolved during composting was measured as described by Michel et al. (1993).

Extraction of atrazine and its metabolites were carried out using the procedure described by Rao et al. (1995). Unextractable radiolabel was measured by combusting 100 mg samples of extracted compost as described by Michel et al. (1995).

RESULTS AND DISCUSSION

Effect of temperature on substrate mineralization. Mineralization of substrate carbon to CO_2 was most effective at 37°C (Figure 3.1). A 10% conversion of biomass to CO_2 was achieved at the end of 84 days of composting at 37°C as compared to a 5.9% conversion at 55°C and 4.6% conversion at 25°C. Statistical analysis showed that the amounts of CO_2 produced at the three temperatures were statistically different. The higher conversion at 37°C suggests higher microbial activity at that temperature. McKinley and Vestal (1985, 1984) reported the greatest microbial activity in compost samples taken from lower temperature areas (25°C-45°C) of sewage sludge windrow composts. Snell (1957) investigated the effect of temperature on the lab-scale composting of ground garbage and reported an optimum temperature of 45°C based on the oxygen uptake rates in the composters. On the other hand, Schulze (1962) observed that temperature was directly related to microbial oxygen uptake rate between 30°C and 70°C during the small-scale composting of garbage. Other researchers have reported composting temperature optima around 55-60°C based on the amount of substrate converted to CO_2, using substrates such as sewage sludge (MacGregor et al., 1981), table scrap (Suler and Finstein, 1977), and municipal refuse (Jeris and Regan, 1973). However, interpretation of these results is difficult due to the variations in the compost substrates and differences in the composting systems. Since the compost substrate used in this study was poplar wood, a slow degrading substrate principally susceptible to fungal attack (Boominathan and Reddy, 1992), the higher conversion seen at 37°C could be an indicator of fungal degradation rather than bacterial degradation.

Effect of temperature on atrazine mineralization. Mineralization of atrazine during the composting of poplar wood as the substrate was minimal at the three different temperatures (Figure 3.2), with an observed mineralization of only about 1%, and did not reflect the differences seen in the conversion of total substrate carbon to CO_2. Also, the radiochemical purity of ^{14}C labeled atrazine was reported as only >98%. Thus there could be a possibility that the $^{14}CO_2$ evolved might not be from ^{14}C labeled atrazine. These results indicate a lack of direct correlation between the mineralization of poplar wood and that of atrazine. Similar results were reported by other researchers (Nair and Schnoor, 1992; Kaufman and Blake, 1970) who observed minimal mineralization of ^{14}C-ring labeled atrazine to $^{14}CO_2$ in soils at 25°C.

Effect of an amendment on substrate mineralization. The rate and extent of conversion of corn cobs to CO_2 at 55°C was about 18% after 84 days of composting, whereas in otherwise identical composters, but with poplar wood as the substrate, <6% mineralization was observed (Figure 3.3).

Effect of amendment on atrazine mineralization. Mineralization of atrazine was minimal either with corn cobs or poplar wood as the substrate (Figure 3.4). For example, about 1% mineralization of atrazine was observed with wood as the substrate but only about half that much mineralization was observed with corn cobs as the substrate. Thus, there was no apparent correlation between the rate and extent of atrazine mineralization and mineralization of corn cobs to CO_2.

Atrazine degradation during composting. Radioactivity distribution data (Figure 3.5) showed that on day zero, ^{14}C was primarily in the chloroform fraction. Samples taken on

day 60, on the other hand, showed a change in the distribution with a greater amount of the ^{14}C in the $NaOH+Na_4P_2O_7$ fraction, which extracted polar metabolites and humic components. For example, 95% of the extracted ^{14}C in the day zero sample from wood (in composters at 55°C), was in the chloroform fraction which extracted atrazine, with 4% in the methanol fraction which extracted non-polar metabolites of atrazine. On the other hand, only 14% was extractable into the chloroform fraction at the end of 60 days of composting, whereas 40% was extractable into the methanol fraction and 15% into the $NaOH+Na_4P_2O_7$ fraction with 28% in bound (unextractable) residues. This general trend towards lesser amounts of radioactivity in the chloroform fraction and greater amounts of radioactivity in the methanol and $NaOH+Na_4P_2O_7$ fractions was seen in the other composts as well. These results suggest that atrazine is being transformed into more polar metabolites and/or is complexing with humic components, as shown recently in the case of 2,4-D composting by Michel et al. (1995), who showed that about 23% of 2,4-D carbon is present in the high molecular weight humate fraction during the composting of 2,4-D with yard trimmings. Our results are further supported by the findings of Winkelmann and Klaine (1991) who showed that, after 180 days incubation with soil microcosms, soil bound residues of atrazine and its metabolites accounted for as much as 60% of the initial radioactivity applied (as atrazine) to the microcosms.

CONCLUSIONS

Mineralization of atrazine during the composting of wood was evaluated at three different temperatures: 25°C, 37°C, and 55°C. Conversion of wood was higher at 37°C than at 25°C or 55°C. Mineralization of atrazine was minimal at all three temperatures. A

comparison of wood as the substrate at 55°C to corn cobs (also at 55°C) showed that a higher percentage (18%) of the carbon from corn cobs was mineralized to CO_2 as compared to that from poplar wood as substrate; however, mineralization of atrazine to $^{14}CO_2$ was minimal (<2%) with either of the substrates.

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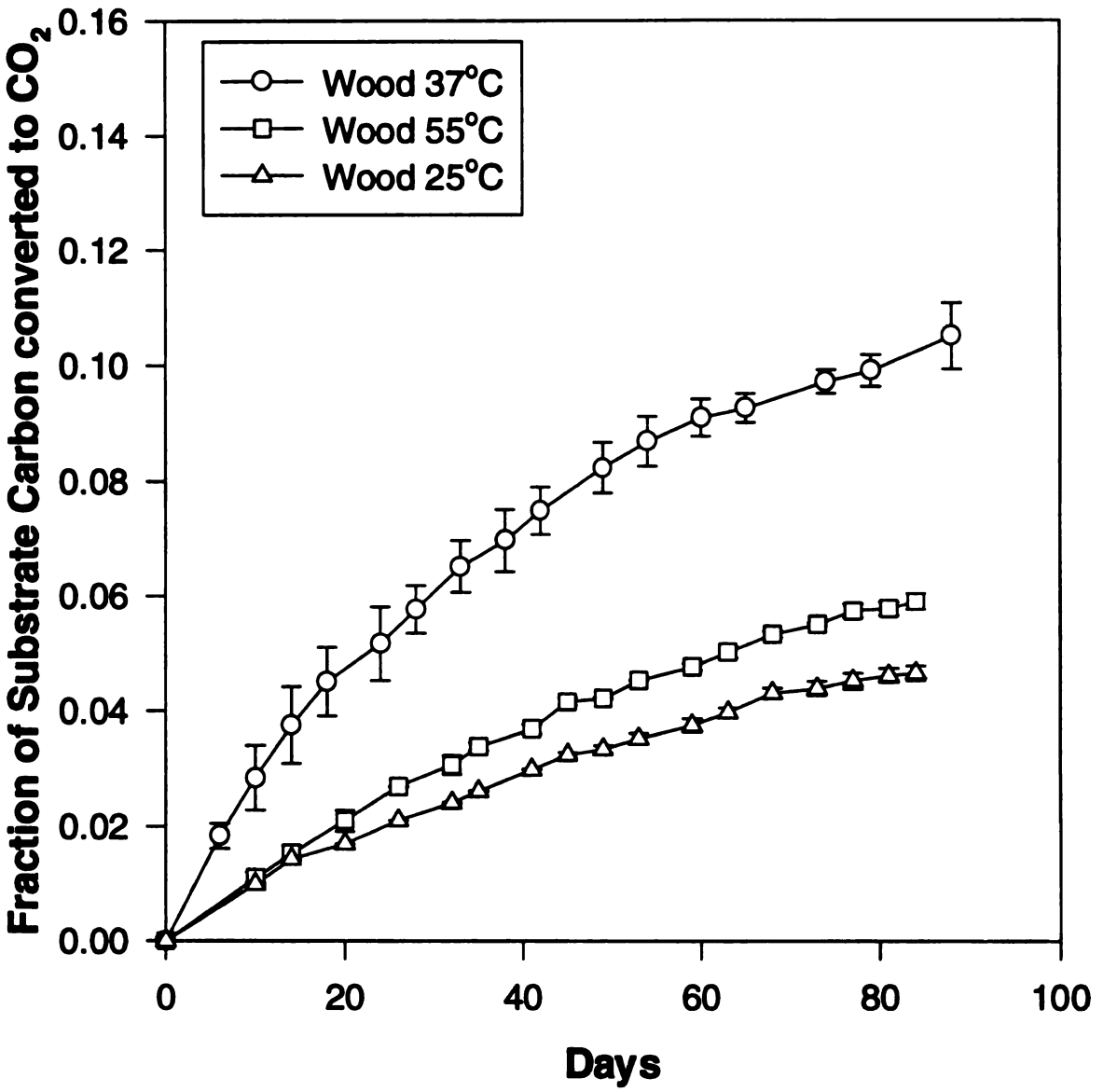

Figure 3.1: Conversion of poplar wood to CO_2 at three temperatures. Values presented are means ± half range for duplicate composters.

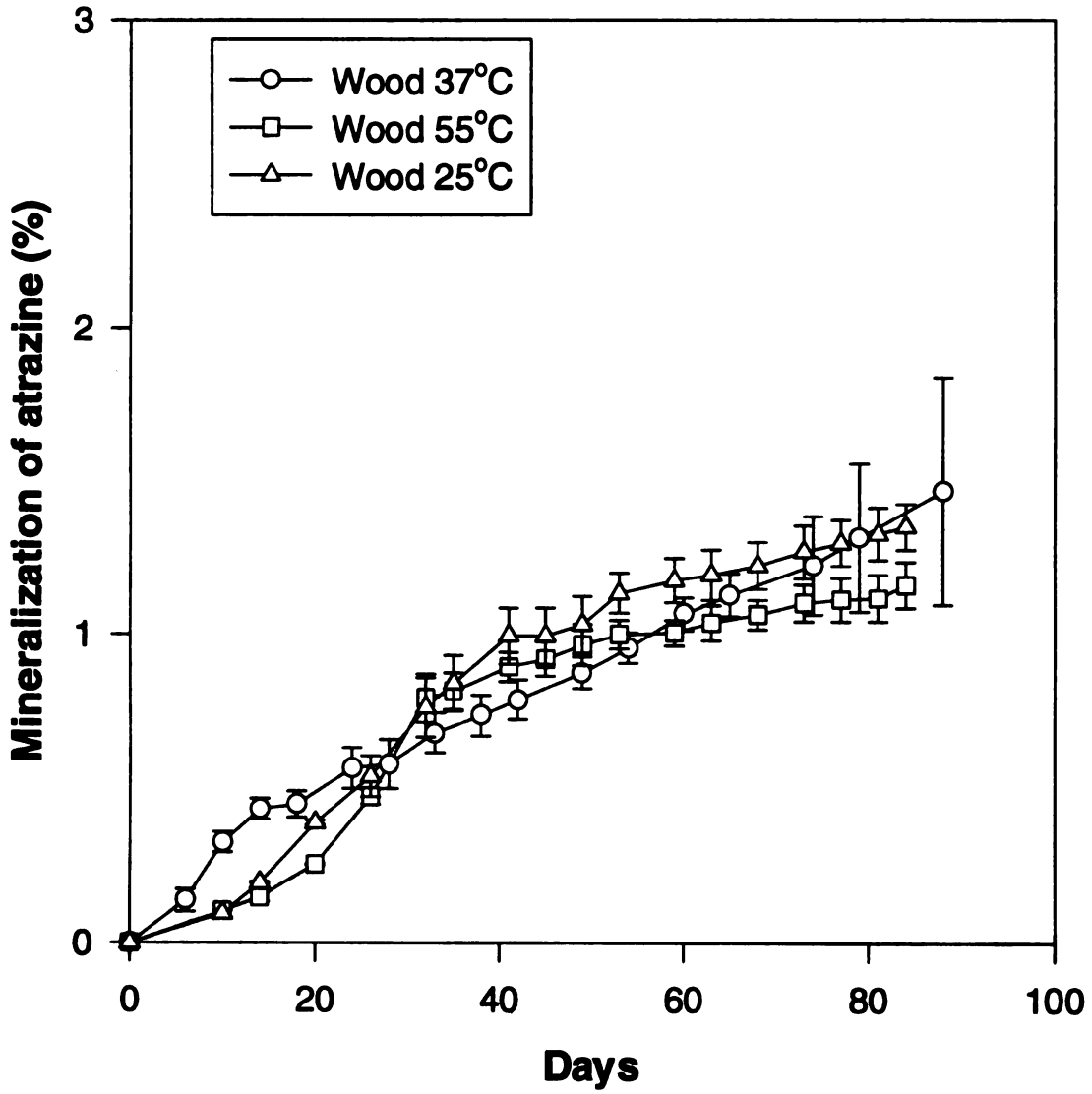

Figure 3.2: Mineralization of ^{14}C-ring labeled atrazine to $^{14}CO_2$ during the composting of poplar wood at three different temperatures. Values presented are means ± half range for duplicate composters.

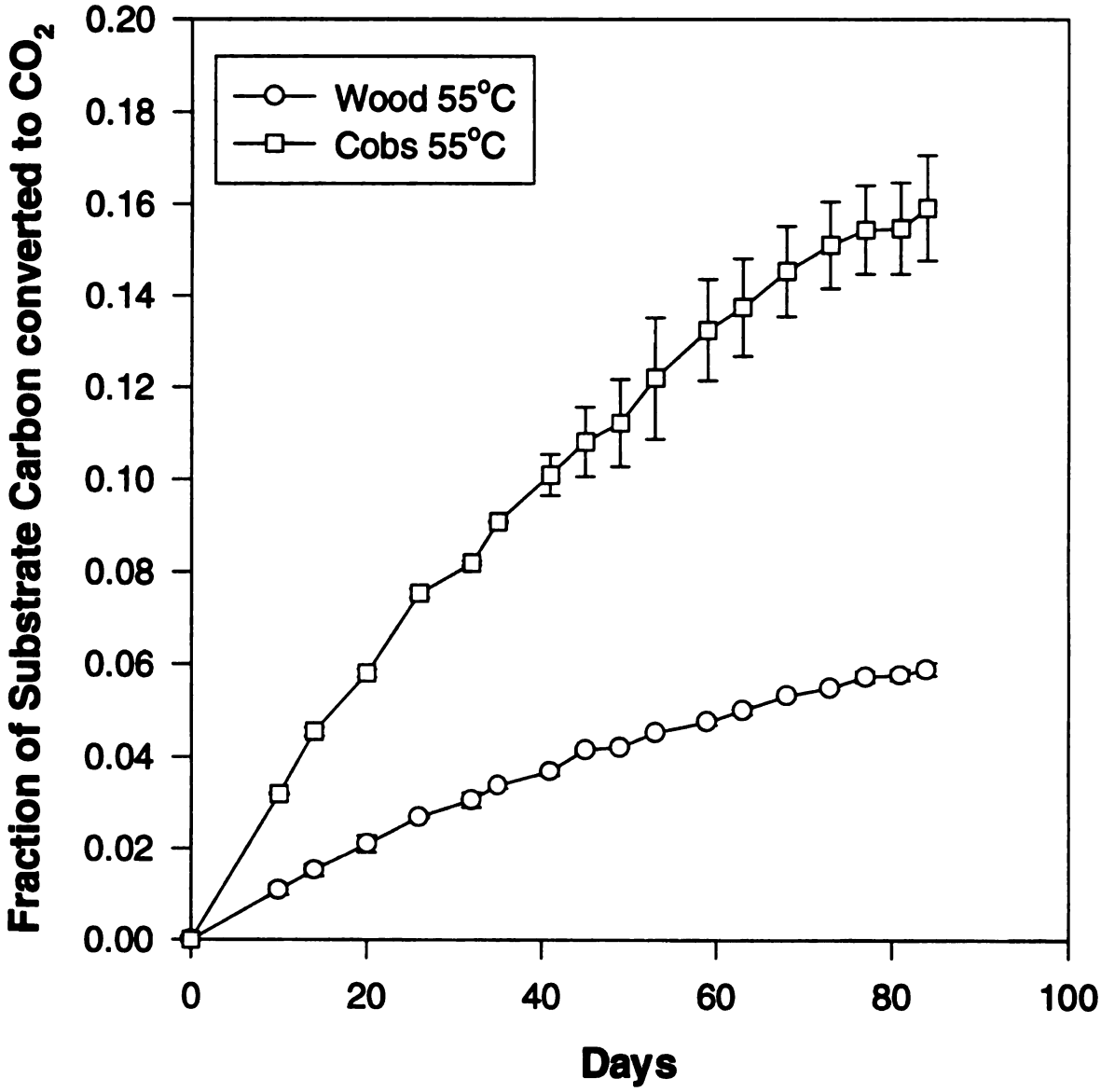

Figure 3.3: Conversion of substrate carbon to CO_2 during composting at 55°C. Values presented are means ± half range for duplicate composters.

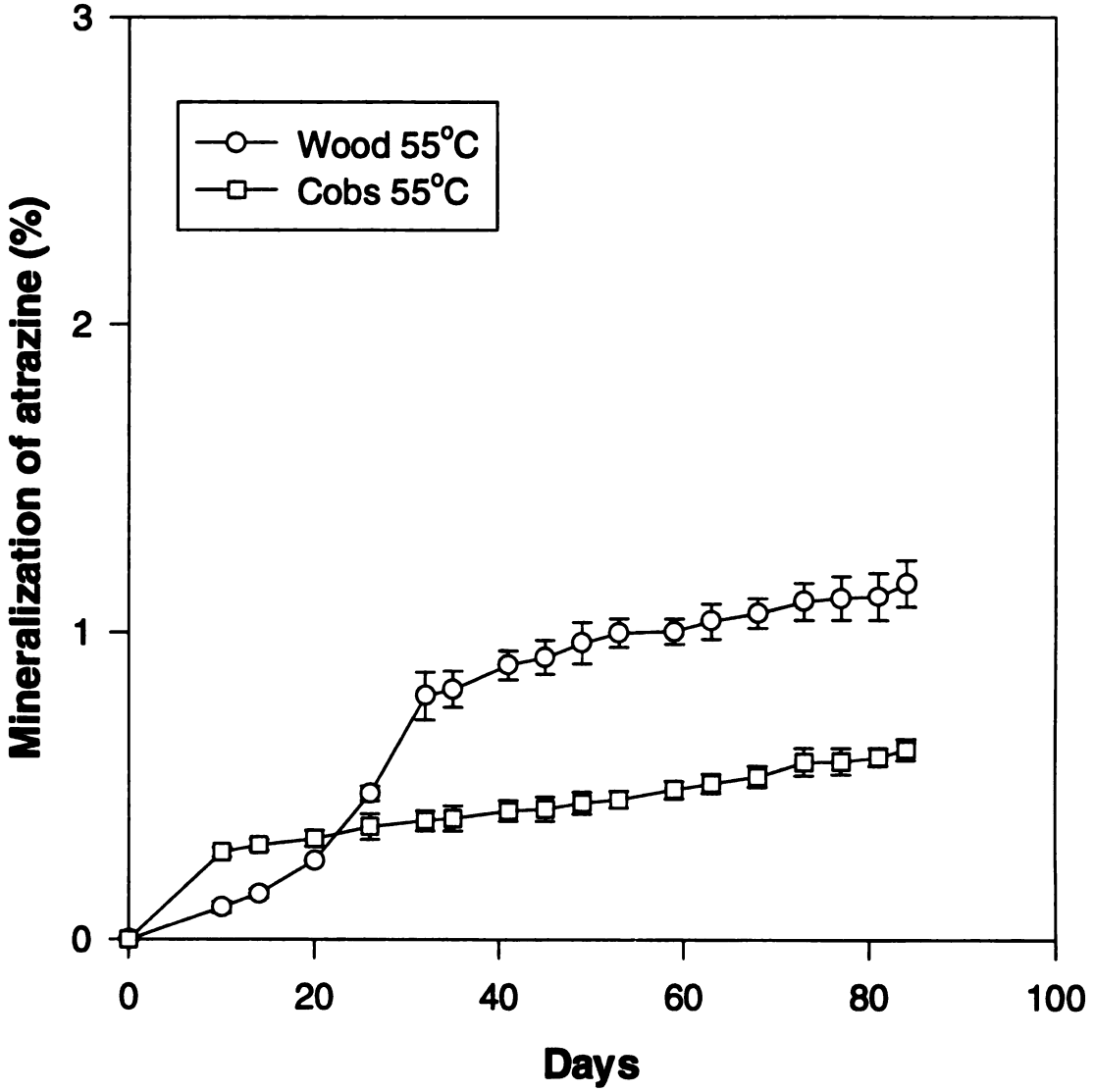

Figure 3.4: Mineralization of ^{14}C-ring labeled atrazine to $^{14}CO_2$ during composting at 55°C. Values presented are means ± half range for duplicate composters.

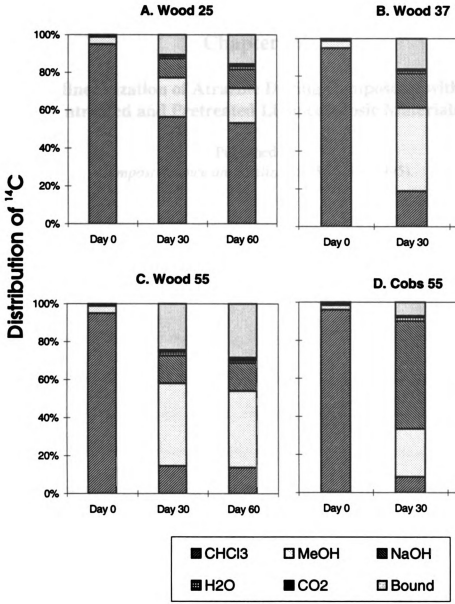

Figure 3.5 Distribution of ^{14}C from compost samples at different time periods in various extraction solvents. Extraction procedures used are described in Materials and Methods. 'NaOH' in the legend box refers to the fraction of ^{14}C radiolabel extracted into the $NaOH+Na_4P_2O_7$ solution. 'Bound' in the legend box refers to the unextracted fraction of ^{14}C radiolabel.

Chapter IV

Mineralization of Atrazine During Composting with Untreated and Pretreated Lignocellulosic Materials

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Abstract

Composting offers a relatively inexpensive and environmentally safe method for the potential bioremediation of pesticide-laden rinsewater. The purpose of this study was to evaluate whether degradation and mineralization of atrazine, a pesticide used extensively in the US, can be enhanced by composting with pretreated lignocellulosic materials as compared to untreated lignocellulosics. Wood that was subjected to steam explosion (STEX wood) or ammonia explosion (AFEX wood), untreated wood (native), and shredded newspaper were selected as the composting substrates. These substrates which differed in composition as determined by the quantitative saccharification and enzymatic hydrolysis techniques, were amended with [U-ring-^{14}C]Atrazine (500 ppm, 5.6 μCi per composter) and composted in 2-liter lab-scale composters. The results showed that the highest rate and extent of total organic matter mineralization to CO_2 was observed with AFEX wood as the substrate, but atrazine mineralization was relatively higher (11%) with paper as the substrate. There was no significant enhancement in atrazine mineralization when composted with the pretreated woods (AFEX wood and STEX wood) as compared to that observed with the native wood. Thus, pretreatment of the wood, which was hypothesised to lead to increased substrate and atrazine mineralization, was seen to have no added effect on atrazine mineralization.

Introduction

A variety of pesticides in common use in agricultural and lawn care applications represents a potential threat to public health and environmental quality. Failure to use proper procedures at pesticide mixing and handling sites and improper disposal of

pesticide laden rinsewater, which can contain from 30 to 2000 mg/L pesticide, can result in contamination of soil, surface water, and groundwater (Myrik, 1990; Norwood, 1990; Toller and Flaim, 1988). Atrazine is one of the most widely used pesticides in the U.S. accounting for about 12% of all the pesticides used in this country (Aspelin et al., 1991). Over 36 million kg of atrazine were applied nationwide in 1990 (Periera and Rostad, 1990). Atrazine and its metabolites are the most frequently detected pesticides in surface waters of the midwestern US (Thurman et al, 1991).

Composting is a relatively inexpensive, easily manageable, and environmentally safe alternative that is increasingly being used in the disposal of municipal solid wastes and yard wastes (Fogarty and Tuovinen, 1991; Michel et al., 1993). Lignocellulosic materials are an ideal choice as a composting substrate because they have been shown to concentrate pesticides from wastewater sources owing to their high sorption characteristics (Toller and Flaim, 1988; Hetzel et al., 1989; Mullins et al., 1993). Furthermore, lignocellulose degrading enzymes may be important for the degradation of pesticides and xenobiotics (Hammel, 1992; Boominathan and Reddy, 1992; Fogarty and Tuovinen, 1991; Bumpus and Aust, 1987). Mullins et al. (1993) proposed that pesticides sorbed on lignocellulosic materials such as steam exploded wood, newspaper, peanut hulls, and peat moss could be degraded to nontoxic products via composting and that this system represents a cost effective and technically uncomplicated approach to treat pesticide laden wastewater. These investigators found peat moss and steam exploded wood to be excellent sorption materials for the pesticides chlorpyrifos and metolachlor, with a nearly complete removal of the pesticide from solution.

Solid-state fermentation of atrazine using bioreactors containing steam exploded wood was shown to decrease solvent extractability of atrazine by 80% within 320 days (Berry et al., 1993). Solid-state fermentation of atrazine using bioreactors containing nutrient enriched peat moss resulted in an 86% disappearance of atrazine at the end of 26 weeks (Mullins et al., 1993). However, the extent of mineralization of atrazine and its fate (mineralization, degradation to polar metabolites, and humification) during composting was not investigated. Also, there is no information on the relative rate and extent of mineralization of atrazine when it was composted with different lignocellulosic substrates. The rate and extent of mineralization of atrazine during composting of lignocellulosic substrates with differing physical and chemical characteristics are presented in this paper. The pretreated substrates were chosen based on the increase in surface area due to the pretreatment (Thompson et al., 1992) and due to the increase in pore size of the substrates leading to better substrate accessibility to enzymes (Grous et al., 1986; Dale and Moreira, 1982). The hypothesis was that pretreatment of the substrate would result in faster conversion of substrate to CO_2 and would also lead to increased mineralization of atrazine in the compost matrix. Also, steam explosion and ammonia explosion are two of the more widely used pretreatment procedures for increasing the accessibility of the carbohydrate polymers of wood to cellulases and hemicellulases (Grous et al., 1986; Dale and Moreira, 1982).

Materials and Methods

Pesticides

[U-ring-^{14}C]Atrazine (specific activity: 7.8 mCi/mmol; purity>98%) was obtained from Sigma Chemicals, St. Louis, MO. Analytical grade atrazine was obtained from Chem Service (West Chester, PA). AAtrex 4L, a commercially available sprayable atrazine emulsion (50% active ingredient), was obtained from Ciba-Geigy (Greensboro, NC).

Compost Substrates

Four substrates were used in this study: untreated poplar wood (Native), ammonia exploded (AFEX) wood, steam exploded (STEX) wood, and shredded newspaper, a lignocellulosic substrate derived from chemi-mechanical treatment of wood. The poplar used in this study was a hybrid grown at the Kellogg Biological Station and was provided by the NSF Center for Microbial Ecology at Michigan State University. The clone is a hybrid between *Populus nigra* and *Populus deltoides* and has been designated *Populus x euramericana* var. *eugenei.* The poplar wood was ground in a Wiley mill through a #10 screen (1.68 mm mesh size) and was used in this form or was subjected to the following pretreatments: 1. steam explosion at 350 psi for 5 minutes (Grous et al, 1986); and 2. ammonia explosion in which the native wood (at 30% moisture, g/g dry wood) was treated for 30 min with anhydrous ammonia (3 kg per kg dry wood) at 50°C and a conventional AFEX explosion was used (Dale and Moreira, 1982). The AFEX wood was provided to us by Dr. Bruce Dale, Texas A&M University, College Station, TX. Newspaper shredded to 3/8" strips, was obtained from Applegate Insulation, Lansing, MI.

Water was added to each lignocellulosic substrate to 70% moisture (g/g wet wt.) and each substrate was loaded into three replicate composters. The composters were amended with [U-ring-^{14}C]Atrazine (5.6 μCi per composter) and AAtrex (500 ppm atrazine/g dry substrate). The inoculum (10%) used for composting was obtained from 10-week-old wood compost piles operated by a large scale composting facility (Hollandia Gardens, Holland, MI).

Composting System

Composting was carried out in the laboratory scale composting system recently described by Michel et al. (1993). In brief, the system consisted of a rubber stoppered 2-liter, wide mouth glass jar with two plastic screens (1cm and 1mm mesh opening) forming a false bottom. Aeration was provided through a hole just below the level of the two screens. CO_2-free, humidified air for the composters was obtained by passing the air through a flask containing 5 N NaOH to remove CO_2 and then through a 5 gallon carboy containing 2.5 gallons of distilled water. The air flow to each of the composters was set at 100 ml/min by means of a needle valve placed just upstream of the composters. The exhaust gas from each composter passed through a polyethylene tube containing polyurethane foam plugs to trap any volatiles and then into two 5N NaOH containers to trap CO_2 present in the exhaust gas. To ensure that the ^{14}C in the NaOH traps was due to $^{14}CO_2$ and not due to volatilized ^{14}C-atrazine or other volatile organics derived from ^{14}C-atrazine, a barium chloride precipitation step was included as previously described (Yadav and Reddy, 1993). The entire system was placed in a temperature controlled room which was maintained at 37°C.

Compost Analysis

Substrate Composition. The quantitative saccharification technique developed by Saeman et al. (1945) was used to determine the cellulose, hemicellulose, and lignin contents of the initial compost substrates. The procedure consisted of hydrolysis of the samples with 72% sulfuric acid for an hour, dilution to a 4% acid solution, and then autoclaving at 121°C for an hour. Sugar concentrations in the hydrolysate were measured using high pressure liquid chromatography (Converse et al., 1989). Glucose obtained from sample hydrolysis was assumed to originate from cellulose and five carbon sugars and mannose were assumed to originate from hemicellulose (Grous et al., 1986; Thompson et al., 1992).

Enzymatic Hydrolysis. Enzymatic hydrolysis of each lignocellulosic substrate used for composting was carried out with cellulase and β-glucosidase using the method of Thompson et al. (1992). Cellulase (Novo CCN 3000, a cellulase from *Trichoderma reesei* with a cellulase activity of 52.1 FPU/ml) and β-glucosidase (Novozym TN 188, isolated from *Aspergillus niger* and having an activity of 588 U/ml) were purchased from Novo Industries (Copenhagen, Denmark). The enzymes were used at a level of 80 units/g dry substrate. Glucose yield from the hydrolysis was calculated as a percentage of the theoretical maximum glucose yield based on the amount of cellulose in the original sample.

CO_2 Evolution. Total CO_2 as well as the amount of $^{14}CO_2$ trapped in the NaOH trap was measured as described by Michel et al. (1993).

Compost Extraction Procedure. Since atrazine and its metabolites exhibit a wide range of polarities and solubilities (Judge et al., 1993), a modified extraction procedure based on

the methods of Smith (1981), FDA (1982), and McCall (1981) was used to determine the distribution of radioactivity in the composts. The extraction procedure was based on the fact that solubility of atrazine is 52000 ppm in chloroform, 18000 ppm in methanol, and 33 ppm in a polar solvent such as water. Samples were periodically taken from the composters and refrigerated until analyzed. The samples were successively extracted with chloroform and methanol (to extract atrazine and its non-polar metabolites), 0.1M $NaOH+Na_4P_2O_7$ solution (to extract polar metabolites and the humic components), and water. Each extraction was carried out with a given solvent till no further ^{14}C was extracted. ^{14}C extracted in each step was calculated by mixing an aliquot of the sample with liquid scintillation cocktail and counting as previously described (Michel et al., 1993).

Thin Layer Chromatography. The chloroform and methanol extracts were concentrated to about 30 µl each and 10 µl was spotted on Silica gel 60, F_{254} TLC plates (Merck, EM Industries, Gibbstown, NJ) to detect the presence of residual ^{14}C-atrazine. The plates were developed using a chloroform-acetone (3:2, v/v) mobile phase (Judge et al., 1993). Radioactive spots on the plate were scanned using a Bioscan System 200 Imaging scanner (Bioscan Inc., Washington, DC).

Results and Discussion

Substrate Composition. The composition of the lignocellulosic materials, obtained by the quantitative saccharification technique, is shown in Table 4.1. The composition of the native wood and AFEX wood were very similar. The STEX wood on the other hand showed a reduced hemicellulose content (i.e. xylan, mannan, arabinan, and galactan) of 8.16%, as compared to 16.17% in native wood, and an increase in the glucan content. The

composition of the shredded newspaper was similar to that of native wood though there was a qualitative difference in the hemicellulose content.

Enzymatic Hydrolysis. Enzymatic hydrolysis data (Figure 4.1) showed that the rate of glucose release as well as the total yield were the greatest with STEX wood (91.5% of the theoretical maximum) which was attributed to the removal of the hemicellulose making cellulose more accessible to cellulase and β-glucosidase (Grous et al., 1986). AFEX wood and newspaper gave similar results with about 50% of the theoretical glucose yield at the end of 48 hours of hydrolysis. Native wood gave the slowest rate and yield of glucose (21.8%). These results agree with previous studies which showed an increase in glucose yield from pretreated wood (Grous et al., 1986).

Total CO_2 Evolution. AFEX wood showed the highest rate and extent of total CO_2 evolution ($^{14}CO_2$ as well as unlabeled CO_2) with 57.7% of the substrate carbon converted to CO_2 at the end of 160 days of composting (Figure 4.2). The total extent of CO_2 production from native wood was only a fraction of that produced by the AFEX wood. Paper and STEX wood showed a relatively long lag period of about 40 days after which there was a substantial increase in the rate of CO_2 evolution. At the end of 160 days of composting, the amounts of CO_2 produced from paper, native wood, and STEX wood were not statistically different, while the amount of CO_2 produced from AFEX wood was statistically different compared to the other three substrates. The higher mineralization observed during the composting of AFEX wood could be due to a higher nitrogen content in the AFEX wood than that in the other substrates. For example, AFEX wood had a C/N ratio of 140:1 while native wood, STEX wood, and paper had C/N ratios of 360:1, 410:1,

and 900:1 respectively. Thus, even though the pretreatments resulted in increased glucose yields as seen from the enzymatic hydrolysis data, these results were not reflected during the composting of these substrates.

Atrazine mineralization and degradation. Mineralization data for ^{14}C-atrazine during composting (Figure 4.3) showed that newspaper supports about 7% mineralization (range: 4 to 11%) after 160 days of composting. By comparison, the AFEX wood, STEX wood, and native wood supported 4.3 %, 1.8%, and 3.3% mineralization, respectively, in the same period. These results are comparable with those of earlier investigators who showed that under aerobic conditions 1.5 % of the s-triazine ring of atrazine was mineralized after 100 days of incubation in soil microcosms receiving 0.37 ppm of atrazine (Nair and Schnoor, 1992). Statistical analysis using the student's t-test (p=0.05) showed that there was no difference in the cumulative $^{14}CO_2$ output at the end of 160 days of composting between any of the substrates. The size of the error bars for atrazine mineralization were much higher in composters with paper as the substrate because activity dropped in one of the three replicate composters from day 50 onwards.

Also, the rate and extent of atrazine mineralization, and mineralization of total carbon to CO_2 were different except in the case of STEX wood (Figure 4.4). This could mean that the mechanisms controlling the conversion of substrate carbon to CO_2 were different from those responsible for the mineralization of atrazine. An observation of the trends in atrazine mineralization (Figures 4.3 and 4.4) showed that atrazine mineralization followed biphasic kinetics with a slower mineralization rate during the first 70 days of composting and a faster rate after this initial period. This could be attributed to a change in

the microbial population during that period or to the triggering of some enzyme system(s) in populations already present in the compost matrix.

Radioactivity distribution data (Figure 4.5) showed that on day zero, ^{14}C was primarily in the chloroform fraction. Samples taken on day 160, on the other hand, showed a change in the distribution with a substantial fraction of the ^{14}C in the $NaOH+Na_4P_2O_7$ fraction. For example, 95% of the extracted ^{14}C in the day zero sample of the AFEX wood compost was in the chloroform fraction, whereas only 0.3% of the ^{14}C was extractable into this fraction in day 160 samples. This general trend towards decreasing amounts of radioactivity in the chloroform fraction and increasing amounts of radioactivity in the $NaOH + Na_4P_2O_7$ fraction was seen in the other three composts as well. These results suggest the transformation of atrazine carbon to more polar metabolites and/or its complexing with humic components as has been recently shown in the case of 2,4-D composting with yard wastes (Michel et al., 1994). Also, the amount of unextractable (bound) radiolabel increased in the compost samples as composting progressed. For example, about 2% of the radiolabel was unextractable in the day zero sample of AFEX wood, whereas 39% was unextractable in day 160 samples. Our results are supported by the findings of Winkelmann and Klaine (1991) who showed that, after 180 days incubation with soil microcosms, soil bound residues of atrazine and its metabolites accounted for as much as 60% of the initial radioactivity applied (as atrazine) to the microcosms.

In support of the above data, the TLC results showed the presence of atrazine on plates spotted with chloroform and methanol extracts from day 0 samples, but not on plates spotted with similar extracts from day 160 samples (data not shown). These results

showed that atrazine disappears completely during composting. Winkelmann and Klaine (1991) also showed that atrazine concentrations decrease exponentially over a period of 180 days in soil microcosms.

Conclusions

The results of this study show complete disappearance of atrazine during 160 days of composting. Up to 11% mineralization of atrazine was observed when it was composted with selected lignocellulosic substrates. No significant differences in atrazine mineralization were observed with untreated wood or pretreated woods as the compost substrates. The time course profiles of organic carbon mineralization to CO_2 generally paralleled atrazine mineralization except with AFEX wood as the substrate. The extent of total organic carbon mineralization observed with a given substrate did not always reflect in the extent atrazine mineralization observed with that particular substrate.

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Table 4.1: Composition of the lignocellulosic substrates used for composting[a]

Substrate	Lignin	Glucan[b]	Xylan[c]	Galactan[c]	Arabinan[c]	Mannan[c]	Total
Native	24.37	43.11	14.12	0.29	0.35	1.41	83.65
AFEX	24.15	42.61	13.09	0.18	0.42	1.65	82.10
STEX	27.88	54.51	6.92	0.24	0.02	0.98	90.55
Paper	28.43	43.68	4.68	1.50	0.96	8.42	87.67

[a]Numbers refer to percentage composition by dry weight of each composting substrate. Native refers to untreated poplar wood; AFEX and STEX refer to ammonia exploded and steam exploded wood, respectively; and paper refers to newspaper.

[b] Derived from cellulose.

[c] Derived from hemicellulose.

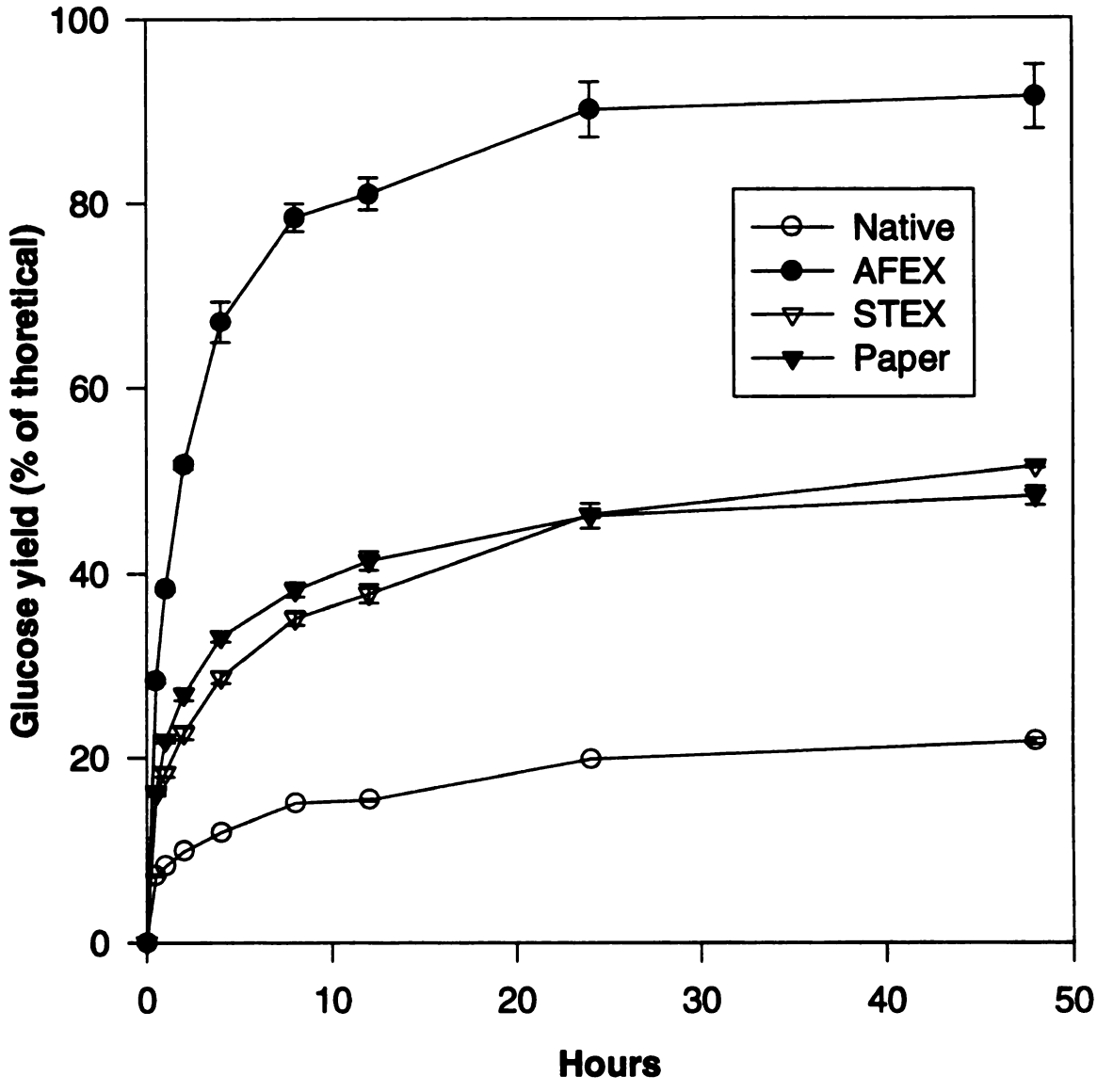

Figure 4.1: Glucose yields from the enzymatic hydrolysis of untreated poplar wood (Native), ammonia exploded (AFEX) wood, steam exploded (STEX) wood, and newspaper. Each substrate was treated with cellulase and β-glucosidase at 80 U/g dry substrate each as previously described (Thompson et al., 1992). Values presented are means ± one standard deviation.

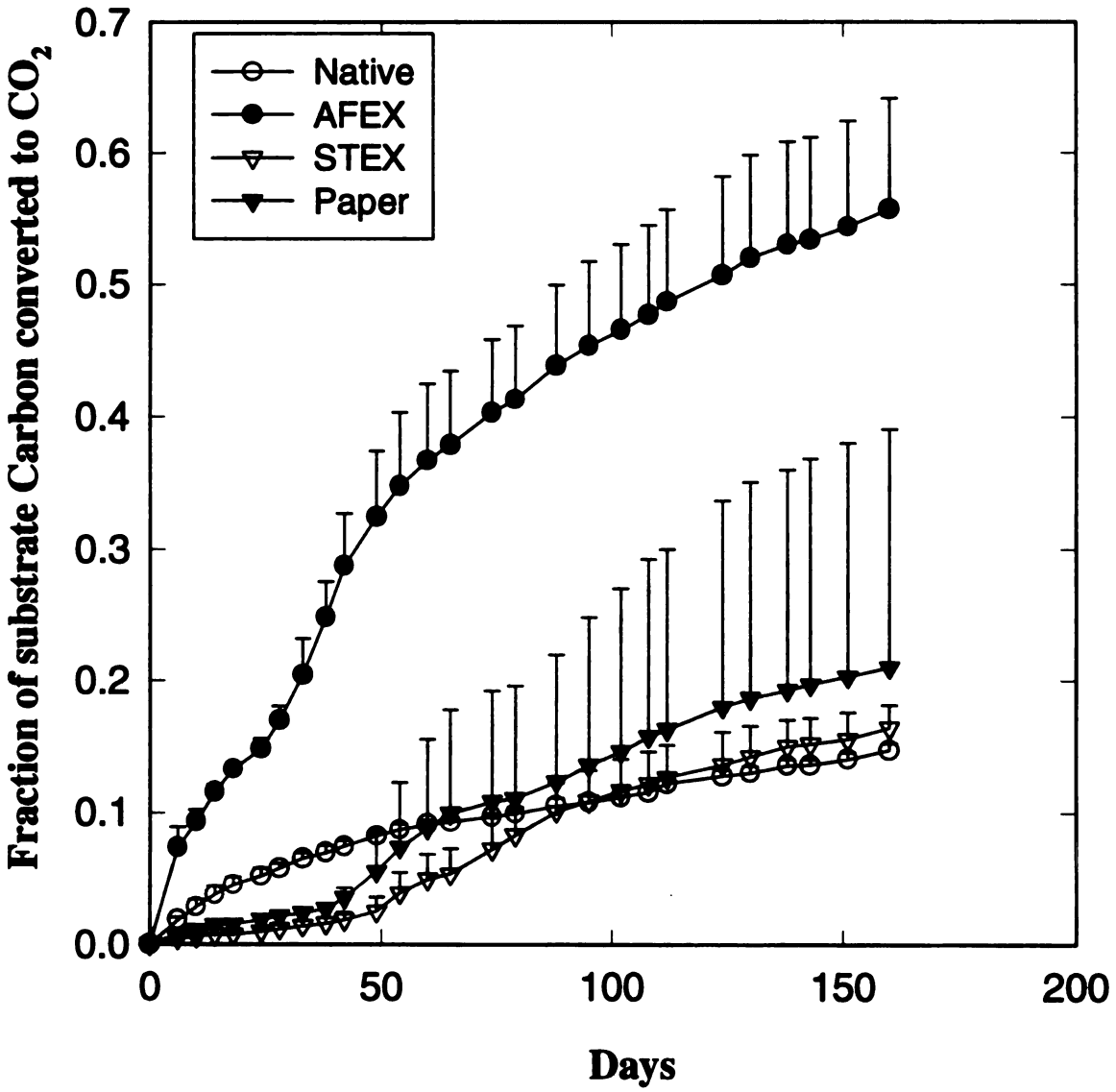

Figure 4.2: Fraction of substrate carbon converted to CO_2. Values presented are means+one standard deviation.

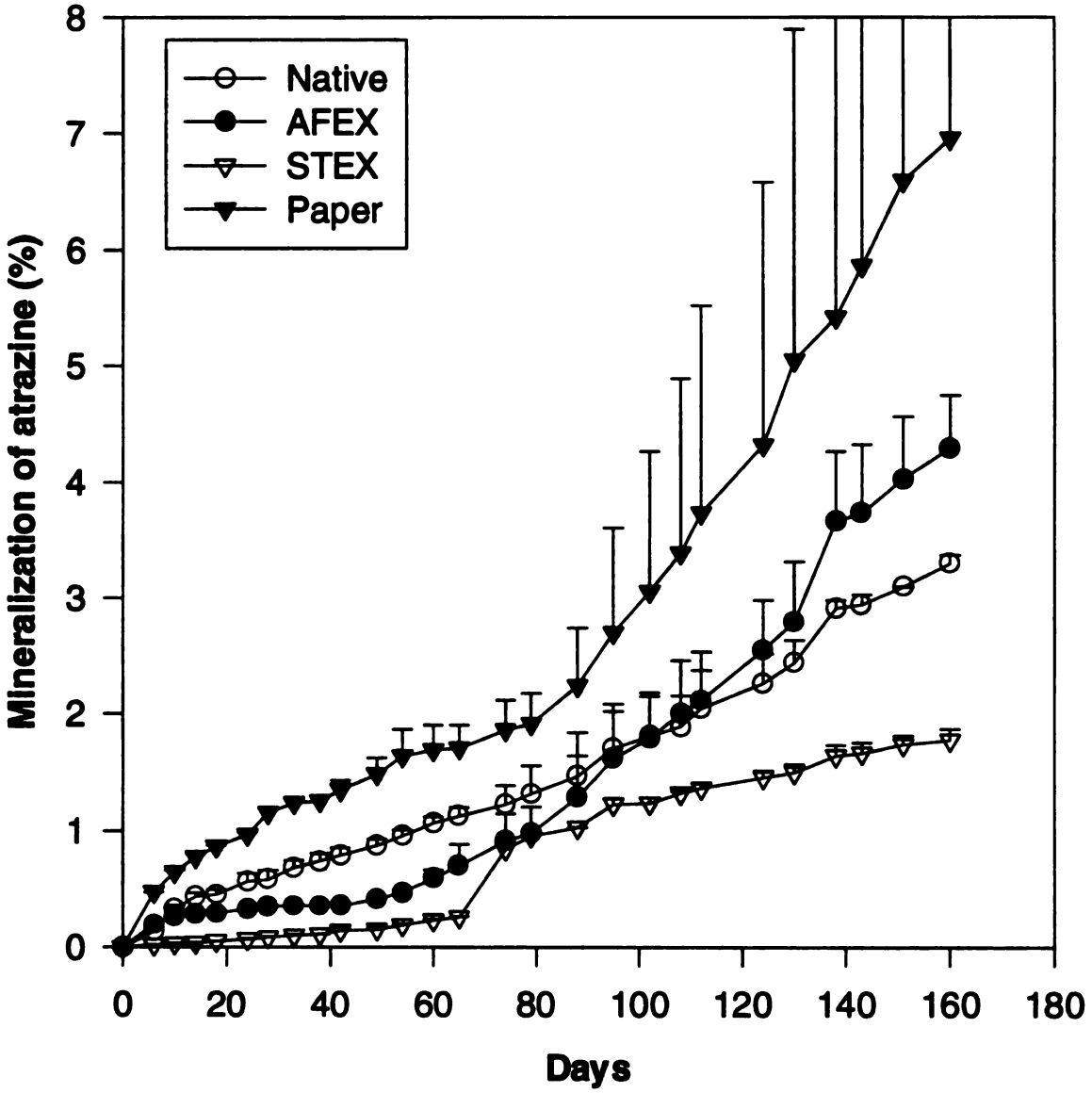

Figure 4.3: Mineralization of ^{14}C-Atrazine to $^{14}CO_2$ during composting with different lignocellulosic substrates. Values presented are means + one standard deviation.

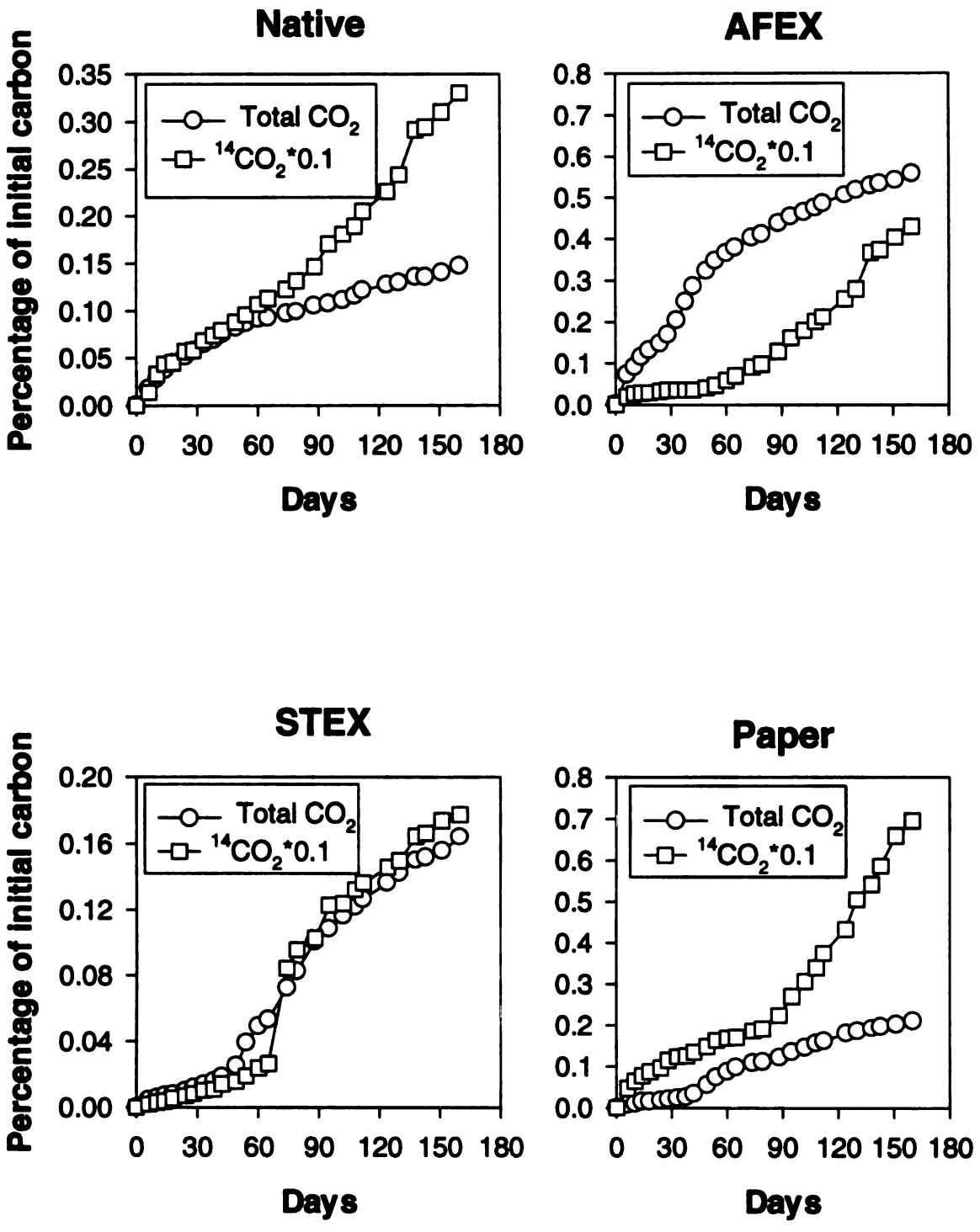

Figure 4.4:.Comparison between total CO_2 production (substrate mineralization) and $^{14}CO_2$ production (atrazine mineralization) for each of the substrates.

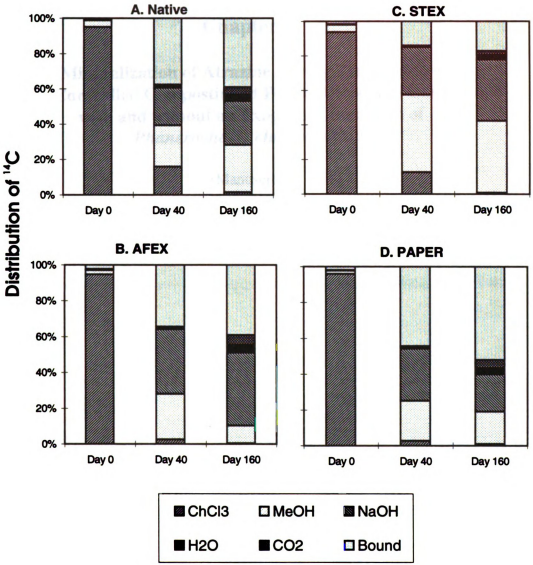

Figure 4.5: Distribution of ^{14}C from compost samples at different time periods in various extraction solvents. Extraction procedures used are described in Materials and Methods. 'NaOH' in the legend box refers to the fraction of ^{14}C radiolabel extracted into the $NaOH+Na_4P_2O_7$ solution. 'Bound' in the legend box refers to the unextracted fraction of ^{14}C radiolabel.

Chapter V

Mineralization of Atrazine During Temperature Controlled Composting of Poplar Wood and Corn with and without an Exogenous Inoculum of *Phanerochaete chrysosporium*

(Manuscript)

ABSTRACT

The effect of an exogenous inoculum of the white-rot fungus *Phanerochaete chrysosporium* on atrazine mineralization during the composting of poplar wood was investigated using 2-liter lab-scale composters. [U-ring-^{14}C]Atrazine was added at 500 µg/g dry substrate (10 µCi) to each composter. *P. chrysosporium* (Strain BKM-F-1767) inoculum was added as an aqueous conidial suspension (9.6 x 10^5 spores/g dry substrate). The addition of *P. chrysosporium* inoculum significantly enhanced mineralization of atrazine, resulting in a 14% mineralization of atrazine in 94 days of composting compared to 1% mineralization observed in the controls without the *P. chrysosporium* inoculum.

INTRODUCTION

Atrazine is one of the most widely used pesticides in the US accounting for about 12% of all the pesticides used in this country (1). Over 36 million kg of atrazine were applied nationwide to agricultural land for the control of annual grasses and broad leaf weeds in 1990 (20). Atrazine and its metabolites are the most frequently detected pesticides in surface waters of the midwestern US (24). Atrazine, along with the others in the *s*-triazine group, is relatively persistent in the environment, with the most heavily substituted and chlorinated *s*-triazine analogs being the least biodegradable (7).

Numerous studies have been conducted to date on the degradation and mineralization of atrazine by microorganisms in pure culture and in situ in soils and other habitats (7,9).

Biodegradation of atrazine has been attributed mainly to fungi (12,13) although recent studies have shown atrazine degradation and mineralization by bacterial species also (3,14,21). Mullins et al. (18) investigated the use of lignocellulosic materials such as steam exploded wood and peat moss as absorbents, for a cost effective approach to treat pesticide laden wastewater. These investigators found peat moss and steam exploded wood to be excellent sorption materials for atrazine, with a nearly complete removal of the pesticide from solution (19). Solid-state fermentation of atrazine using bioreactors containing nutrient enriched peat moss resulted in an 86% disappearance of atrazine at the end of 26 weeks (18). In a recent study, Rao et al. (22) reported upto 11% mineralization of atrazine in 160 days during the composting of lignocellulosic substrates.

White rot fungi, as exemplified by *Phanerochaete chrysosporium*, were shown to be efficient degraders of lignin (a complex, heterogeneous aromatic polymer) in wood. Lignin modifying enzymes of these organisms are relatively non-specific and were shown to fortuitously degrade a variety of chloroaromatic environmental pollutants such as polychlorinated biphenyls and dioxins (5,6,8,23). Hickey et al. (11) recently investigated the potential of *P. chrysosporium* for degrading atrazine in contaminated soils as well as in liquid cultures in the laboratory, and reported insignificant mineralization of ring labeled atrazine to $^{14}CO_2$. Mougin et al. (17) observed a 48% decrease of the initial atrazine (0.43 ppm) after 4 days of incubation with *P. chrysosporium* but they also failed to show significant mineralization of ^{14}C-ring labeled atrazine to $^{14}CO_2$. However, the potential of *P. chrysosporium* to mineralize atrazine during the composting of lignocellulosic materials such as wood has not been investigated before. In this study, we investigated the

mineralization of atrazine during the composting of poplar wood with and without an exogenous inoculum of *P. chrysosporium*.

MATERIALS AND METHODS

Pesticides. [U-ring-^{14}C]Atrazine (specific activity: 25 mCi/mmol; purity>98%) was obtained from Sigma Chemicals, St. Louis, MO. AAtrex 4L, a commercially available sprayable atrazine emulsion (50% active ingredient), was obtained from Ciba-Geigy (Greensboro, NC). Analytical grade atrazine was obtained from Chem Service (West Chester, PA).

Compost Substrates. The poplar wood used in this study was provided by the NSF Center for Microbial Ecology at Michigan State University, and is a hybrid between *Populus nigra* and *Populus deltoides* and has been designated *Populus x euramericana* cv. Eugenei. The poplar wood was ground in a Wiley mill through a #10 screen (1.7 mm mesh size) and adjusted to 70% moisture (g/g wet wt.) by adding distilled water. Coarsely ground corn, obtained from a local pet supply store (Soldan's Pet Supplies, Lansing, MI), was adjusted to 50% moisture and used as an amendment to the wood (1:1 w/w) in one experiment. Wood and wood amended with corn were each loaded into two sets of duplicate composters to give 100 g dry substrate per composter. All the composters were amended with [U-ring-^{14}C]Atrazine (10 μCi per composter) and AAtrex (500 μg atrazine/g dry substrate). Initial carbon content of the substrates was determined using a Leco Carbon Analyzer (Model #598-550, Leco Inc., St. Joseph, MI) by the Michigan State University Soil Testing Laboratory. These values were also verified using the

quantitative saccharification procedure described elsewhere (22).

Compost Inoculum. Inoculum (10% w/w) for all composters came from 10-week-old compost piles operated by a large scale composting facility (Hollandia Gardens, Holland, MI). In addition, *P. chrysosporium* (Strain BKM-F-1767) was added as conidial spores at the rate of 9.6×10^5 spores/g dry substrate to one set of composters with wood alone and another set of composters with wood and corn. Conidial suspensions were prepared as previously described (4).

Composting System. Composting was carried out in the laboratory scale composting system recently described by Michel et al. (16). In brief, the system consisted of a rubber stoppered 2- liter, wide mouth glass jar with two plastic screens (1cm and 1mm mesh opening) forming a false floor. Aeration was provided through a hole just below the level of the two screens. CO_2-free, humidified air for the composters was obtained by passing the air through a flask containing 5 N NaOH to remove CO_2 and then through a 5 gallon carboy containing 2.5 gallons of distilled water. The air flow to each of the composters was set at 100 ml/min by means of a needle valve placed just upstream of the composters. The exhaust gas from each composter passed through polyurethane foam plugs to trap any volatiles and then into two 5N NaOH containers to trap CO_2 present in the exhaust gas. To ensure that the ^{14}C in the NaOH traps was due to $^{14}CO_2$ and not due to volatilized ^{14}C-atrazine or other volatile organics derived from ^{14}C-atrazine, a barium chloride precipitation step was included as previously described (27). The entire system was placed in a temperature controlled room which was maintained at 37°C since *P. chrysosporium* is known to grow optimally at 37°C-40°C (5,6,8).

CO_2 Evolution. Total CO_2 trapped in the NaOH traps during composting was measured by titrating an aliquot of the NaOH solution against standardized HCl. The procedure involved removal of a 0.5 ml aliquot of the NaOH solution, dilution with 1 ml of water, and precipitation of Na_2CO_3 with 3 ml of a 70 g/l solution of $BaCl_2$. 0.5 ml of this diluted solution was then titrated against standardized HCl. The titration value obtained was used to calculate the amount of CO_2 trapped in the test tubes. The amount of $^{14}CO_2$ trapped was measured by mixing 0.2 ml of the NaOH solution with 0.8 ml water and 15 ml of scintillation cocktail (Safety-Solve, Research Products International Corp., Mount Prospect, IL) in 20 ml scintillation vials. These were then counted using a scintillation counter (Tri-Carb 1500, Packard Instrument Co., Downers Grove, IL) after letting the samples sit overnight to eliminate chemiluminescence effects.

RESULTS AND DISCUSSION

Substrate mineralization. The evolution of CO_2 from the composters with wood as substrate with and without *P. chrysosporium* inoculum, is shown in Figure 5.1. The rate of evolution of CO_2 was much higher in the composters inoculated with *P. chrysosporium*, as compared to the composters without the *P. chrysosporium* inoculum. For example there was 61% conversion of the initial substrate carbon to CO_2 at the end of 94 days of composting in composters with *P. chrysosporium* as compared to a 10% conversion observed in the controls. The increased rate of mineralization observed in the composters inoculated with *P. chrysosporium* (after the first 15 days of composting) was also

accompanied by a visible colonization of the substrate by *P. chrysosporium*. It is of interest that in experiments in which *P. chrysosporium* was added to poplar wood as a mycelial blend (Figure 5.2), rather than as a conidial suspension (Figure 5.1), no appreciable difference in the conversion of biomass to CO_2 was seen in the composters with and without the *P. chrysosporium* inoculum.

Atrazine mineralization. Mineralization patterns of atrazine (Figure 5.3) were similar to the mineralization patterns of wood seen in Figure 5.1. ^{14}C-atrazine mineralization to $^{14}CO_2$ was 14% at the end of 94 days of composting in composters with wood and *P. chrysosporium* as compared to a 1% mineralization observed in the controls with wood alone. These results are contrary to the findings of Hickey et al. (11) who investigated the potential of *P. chrysosporium* to bioremediate atrazine-contaminated soils in laboratory studies. They found that $^{14}CO_2$ production from soils with ^{14}C-ring labeled atrazine was insignificant. They also reported that atrazine was not metabolized by *P. chrysosporium* grown in liquid cultures. On the other hand, Mougin et al. (17) observed a 48% decrease of the initial atrazine (0.43 ppm) in liquid cultures of *P. chrysosporium* within the first 4 days of incubation. However, they found only mineralization of the ethyl side chain of atrazine but no mineralization of the atrazine ring. Kaufman and Blake (12) also reported mineralization of the ethyl side chain but no mineralization of the atrazine ring in their study on degradation of atrazine by soil fungi. The evolution of $^{14}CO_2$ from ^{14}C-ring labeled atrazine observed in our study clearly indicates mineralization of atrazine by ring cleavage, a phenomenon not observed by these researchers. The use of wood, the natural substrate for *P. chrysosporium*, may account for the observed mineralization of

atrazine by ring cleavage in our study.

Other researchers have reported mineralization of atrazine by ring cleavage though the amounts of atrazine loaded initially were very low. Assaf and Turco (2) found a 17% mineralization of atrazine (10 ppm initial concentration) in soils after 90 days of incubation. Mineralization levelled out at 39% at the end of 326 days of incubation. Levanon (13) reported a 29% conversion of ring labeled atrazine (1 ppm) to $^{14}CO_2$ in 32 days of incubation in soils. This amounts to atrazine conversion to CO_2 of 3.9 ppm observed by Assaf and Turco (2) and 0.29 ppm by Levanon (13). In comparison, our results show mineralization of 70 ppm of the initially loaded atrazine to CO_2 after 94 days of incubation.

Effect of corn amendment on substrate mineralization. The effect of addition of corn as an amendment to the wood is presented in Figures 5.4 and 5.5. The high rate and extent of conversion of biomass to CO_2 might be attributable to the fact that corn (due to its starch content) is a readily utilizable substrate (Figure 5.4). The addition of *P. chrysosporium* to composters with wood and corn did not result in an increase in mineralization of the substrate carbon to CO_2. The conversion of substrate to CO_2 in the composters with an added inoculum of *P. chrysosporium* (57%) was statistically different compared to that in composters without the inoculum(64%).

Effect of corn amendment on atrazine mineralization. Mineralization of atrazine at the end of 94 days of composting in reactors containing wood + corn and receiving a *P. chrysosporium* inoculum, was not statistically different ($p = 0.05$) from that observed in identical reactors not receiving the *P. chrysosporium* inoculum. Thus, there was no

apparent correlation between the rate and extent of atrazine mineralization and mineralization of total carbon to CO_2.

Previous research has been inconclusive on the effect of energy sources and/or nutrients on the degradation of atrazine. Assaf and Turco (2) reported that atrazine degradation in soils amended with mannitol as a carbon source, and with urea as a nitrogen source at levels of 10-80 mg/kg, was similar to the degradation observed in unamended soils. On the other hand, McCormick and Hiltbold (15) observed that the rate of atrazine decomposition was increased by the addition of glucose to soils. Wagner and Chahal (26) also found that atrazine degradation was accelerated by the presence of glucose. Hance (10) reported that the addition of straw, inorganic salts, or a combination of both approximately doubled the rate of atrazine degradation in soils. Our results showed much lower mineralization of atrazine in composters containing wood + corn and inoculated with *P. chrysosporium* as compared to that observed in composters containing wood alone and inoculated with *P. chrysosporium*. This could be due to the fact that the composters amended with corn were colonized rapidly by a *Mucor* species, a starch utilizing fungus, in the first week of composting (data not shown). Thus the addition of corn as amendment to the wood served to select for a *Mucor* sp., a starch utilizing fungus, while growth of the wood degrading fungus, *P. chrysosporium* was inhibited in these composters. The difference in atrazine mineralization seen in the composters containing wood and those containing wood + corn could be attributed to the difference in the enzyme systems of the fungi colonizing the compost substrates.

In conclusion, the results of this study show that the addition of an exogenous

inoculum of *P. chrysosporium* to composters with poplar wood as the substrate resulted in mineralization of 14% of the initial atrazine in 94 days of composting as compared to 1% mineralization observed in the controls. Mineralization of atrazine was considerably lower (3.8% mineralization in 94 days) in composters receiving wood, corn and *P. chrysosporium* inoculum.

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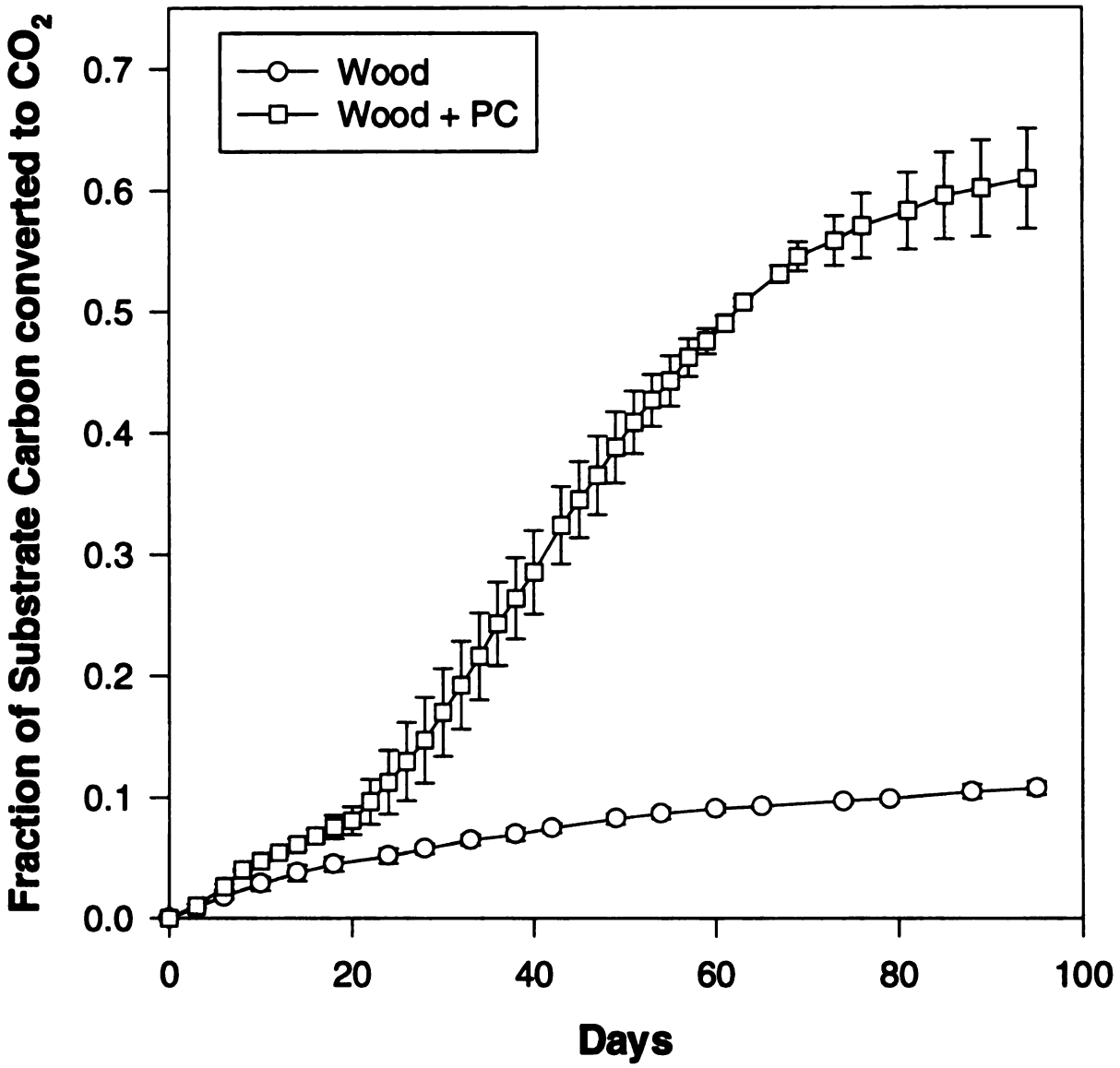

Figure 5.1: Effect of addition of a spore inoculum of *P. chrysosporium* (PC) to poplar wood. Composting was carried out at 37°C. Values presented are means ± half range for duplicate composters.

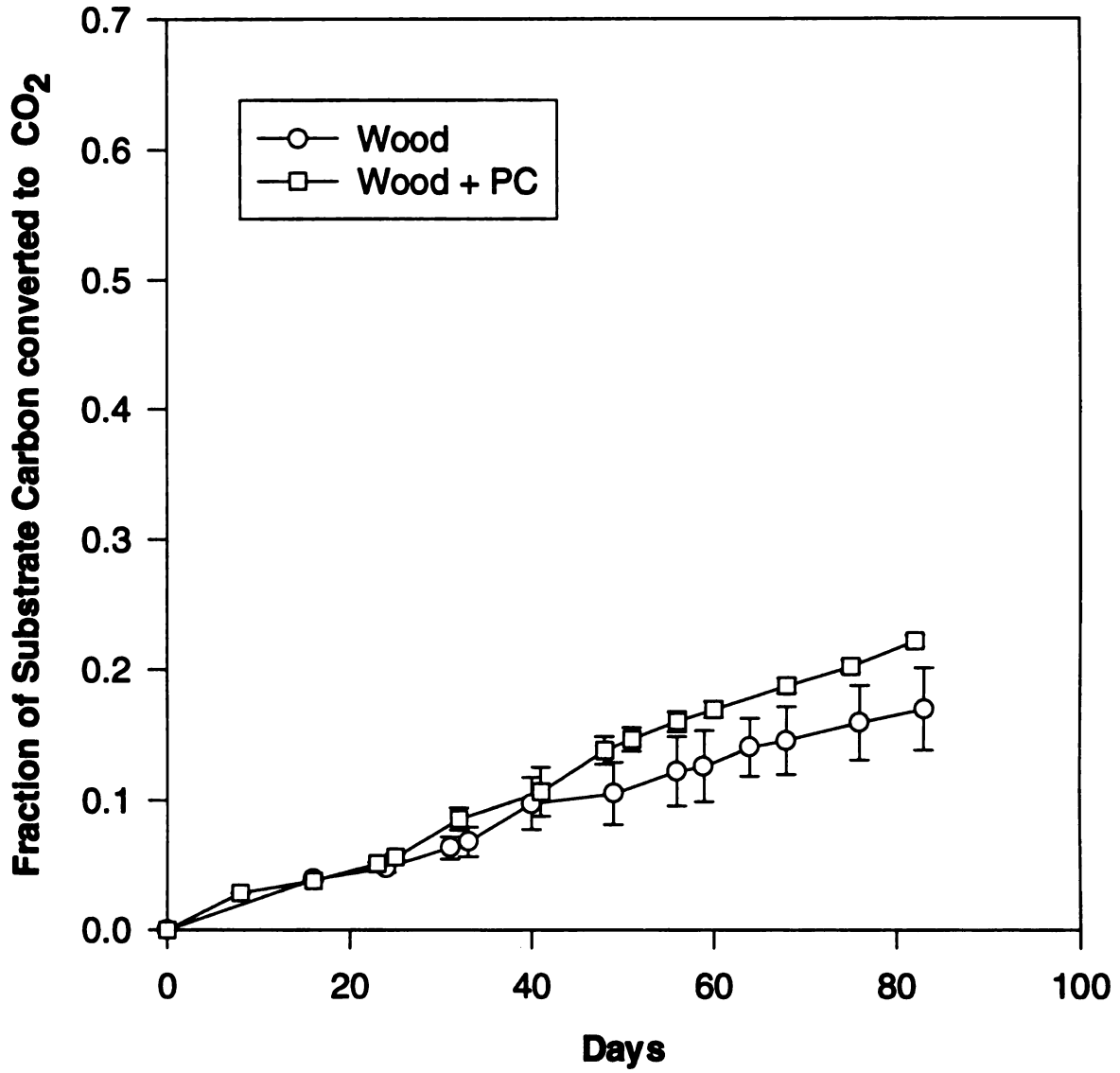

Figure 5.2: Effect of addition of an exogenous inoculum of *P. chrysosporium* (PC) in the form of blended mycelia to poplar wood. Composting was carried out at 37°C. Values presented are means ± half range for duplicate composters.

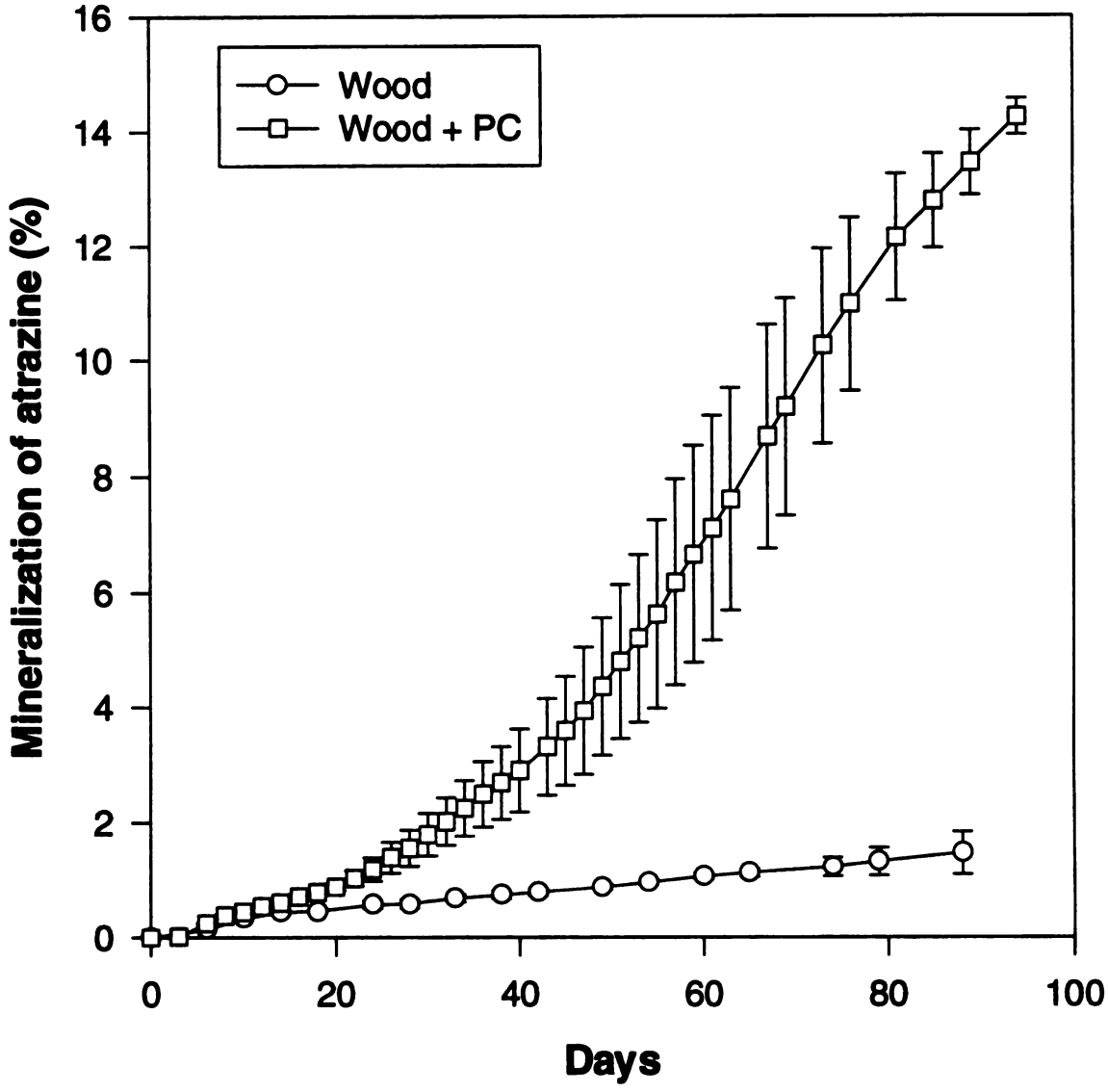

Figure 5.3 Mineralization of ^{14}C-ring labeled atrazine to $^{14}CO_2$ during the composting of poplar wood with and without the addition of an exogenous inoculum of *P. chrysosporium* (PC). Values presented are means ± half range for duplicate composters.

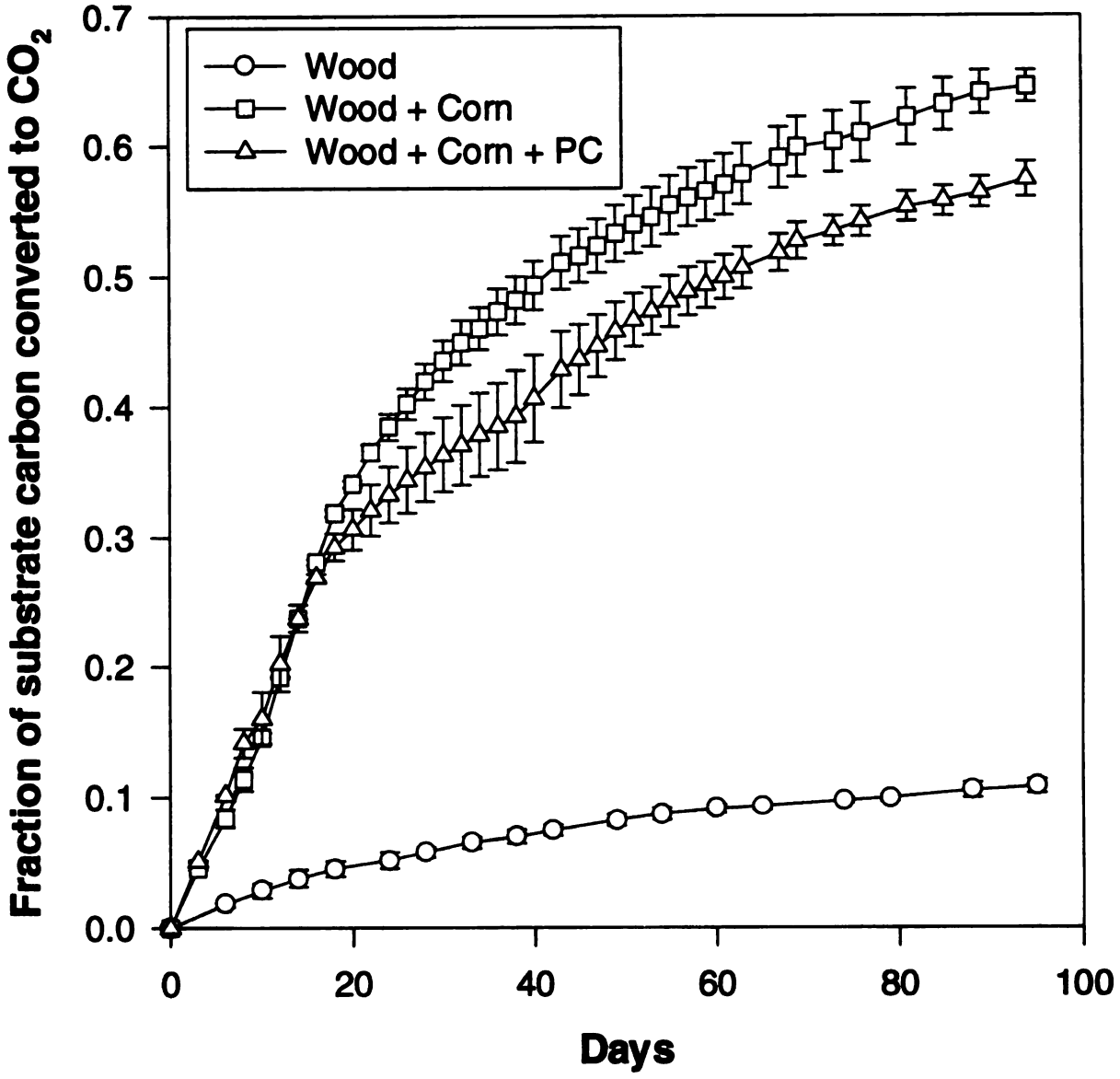

Figure 5.4: Fraction of initial substrate carbon converted to CO_2 during the composting of poplar wood with and without an amendment of corn at 37°C. Values presented are means ± half range for duplicate composters.

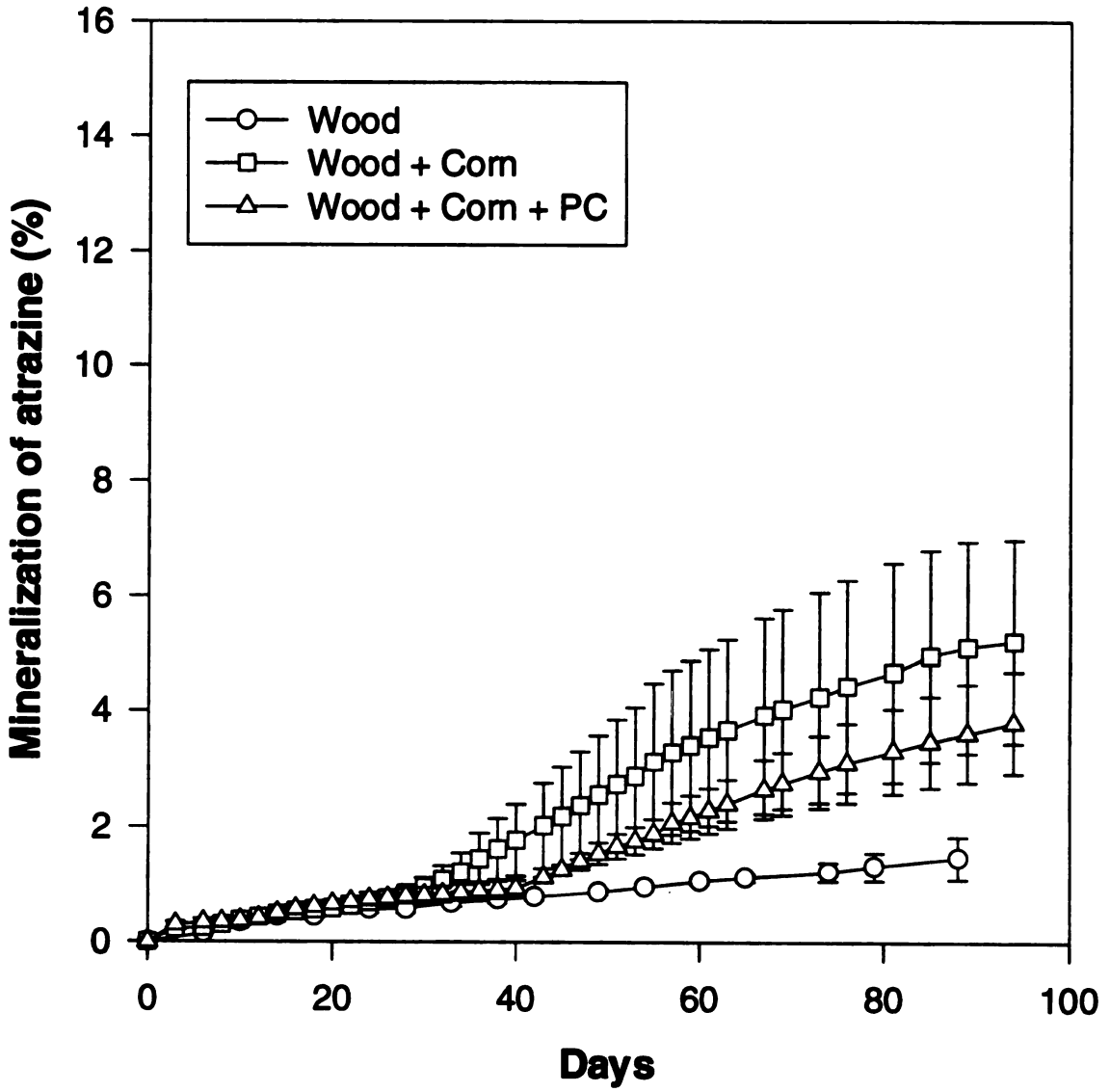

Figure 5.5: Mineralization of ^{14}C-ring labeled atrazine to $^{14}CO_2$ during the composting of poplar wood with and without an amendment of corn. Values presented are means ± half range for duplicate composters.

Chapter VI: Preliminary Process Design and Modelling

Introduction:

The objective of this study was to evaluate a scheme for the disposal of pesticide contaminated water and pesticide rinsewater. From the results of the previous chapter it is evident that co-composting the pesticide with lignocellulosic materials is an effective method for the disposal of the pesticide. This chapter considers the commercial implementation of a co-composting process for the disposal of the entrained pesticide. A theoretical model was also developed to explain the mineralization of poplar wood and atrazine during composting with an inoculum of *P. chrysosporium*.

Process Design:

A schematic diagram of the conceptual process for the disposal of pesticide-contaminated water by co-composting with lignocellulosic materials is shown in Figure 6.1. The entire process from the point of generating/receiving the pesticide-contaminated water to the co-composting of the pesticide with the lignocellulosic substrate is a very simple one with respect to equipment considerations. Mixing of the contaminated water and the composting substrate is very easily achieved by spraying the water on the substrate in the required amounts to achieve the desired pesticide concentration and moisture content in the composting substrate. The composting process itself requires a minimum of equipment for the completion of the process, and includes equipment that control the temperature of the compost windrow to the desired set point. In our case the desired set point would be the optimum temperature for the growth of *Phanerochaete chrysosporium*.

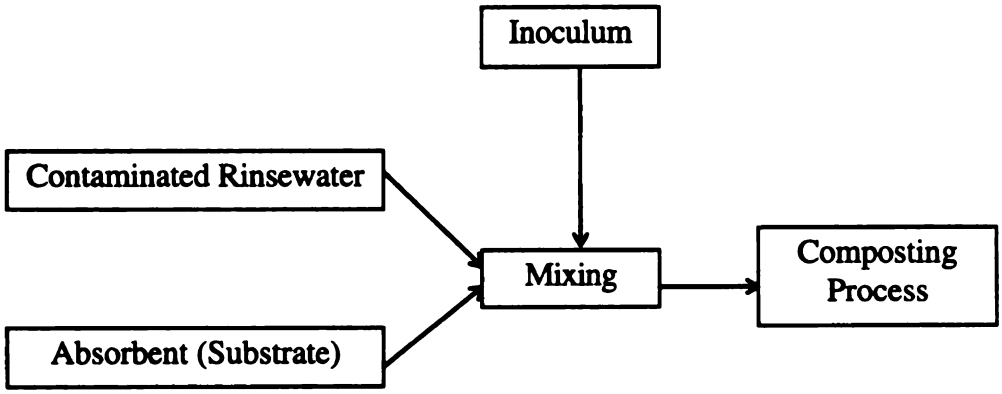

Figure 6.1: Conceptual co-composting process

Temperature control of the compost windrow can be achieved in a couple of ways. One would be by controlling the windrow configuration (windrow length, width, and height), thus allowing for the regulation of conductive heat loss from the compost pile. This option is not feasible in our case since conduction accounts for only 2.4% of all heat loss in field scale composters, the other 97.6% occuring due to evaporative cooling and sensible heat rise of air in the pile (Hogan et al., 1989). Thus a better alternative to controlling the pile temperature would be by the control of aeration through the windrow with the objective of removal of excess heat (above the desired set point) by evaporation of moisture from the composter.

Assuming that composting follows first order reaction kinetics, we get the following equation from a mass balance of the composting process:

$$\frac{dm}{dt} = k(m_t - m_e) \qquad (1)$$

where:

m_t = mass of composting material at time t

m_e = amount of uncomposted material at end of composting process

k = decompostion rate

The theoretical air flow rate required to maintain a constant temperature is then:

$$q = \frac{(-\Delta h_c)k}{(\Delta H_{air})} \qquad \frac{kg_{air}}{kg_c day} \qquad (2)$$

where:

Δh_c = heat of combustion

ΔH_{air} = enthalpy difference of inlet and outlet air

kg_c = amount of compostable material

Total air flow at time t can be calculated from the following equation:

$$Q_t = \frac{(m_t - m_e)q}{\rho_a} \qquad (3)$$

where:

Q_t = total air flow, m^3/day

ρ_a = density of air, kg_{air}/m^3

Fan power required to maintain this air flow:

$$P = \frac{Q \cdot \Delta p}{\varepsilon} \qquad \frac{(m^3/s)(Pascals)}{efficiency} \tag{4}$$

where:

P = power (watts)

Δp = pressure drop across the compost pile

ε = efficiency of the fan

and pressure drop is given by the equation (Higgins, 1982):

$$\Delta p = a \cdot \left(\frac{Q}{A}\right)^n \cdot d \qquad (\text{mm } H_2O) \tag{5}$$

where:

A = floor area of compost pile (m^2)

d = depth of compost pile (m)

a,n = parameters

We can use the following values to calculate the fan power required:

Parameter	Value	Source
k	0.026 day^{-1}	Experimental data
$-\Delta h_c$	20 MJ/kg	Keener et al. (1993)
Air enthalpy (inlet)[a]	62 KJ/kg	Perry (1984)
Air enthalpy (outlet)[b]	184 KJ/kg	Perry (1984)
m_o	3000 kg	Assumption
m_e	1000 kg	Experimental Data
Air density	1.2928 kg/m^3	Perry (1984)
Floor area	20 m^2	Assumption
Windrow depth	2 m	Assumption
a	1.23×10^{-5}	Keener et al. (1993)
n	1.55	Keener et al. (1993)

a - assuming inlet air at 21°C and 50% relative humidity

b - assuming outlet air at 40°C and 100% relative humidity

Using the values listed above:

$q = (0.026)(20{,}000)/(184-62)$

$= 4.262\ kg_{air}/kg/day$

Initial air flow required would be:

$Q_o = (3000\text{-}1000)(4.262)/1.2928$

$= 6593.9\ m^3/day$

$= 0.0763\ m^3/s$

Pressure drop across the compost pile would be:

$\Delta p = (1.23 \times 10^{-5})(2)(6593.9/20)^{1.55}$

$= 0.1968$ mm H_2O

$= 1.93$ Pascals

Initial fan power required:

Fan power $= (0.0763)(1.93)/0.6$

$= 0.245$ Watts

$= 1.83 \times 10^{-4}$ hp

This would be the maximum power requirement since the amount of compostable material would decrease with time and also assuming that the maximum rate of decomposition would be in the initial stages of composting. Assuming cost of electricity to be 6 cents/kWhr, the power requirement cost for the process would be quite minimal (about 1c/month).

Process Model:

The mineralization of wood and atrazine during composting with an inoculum of *P. chrysosporium* observed in the experiments of Chapter V (see Figure 6.2) were modelled as follows. Growth of biomass was modelled using the Verlhurst and Pearl equation (Bailey and Ollis, 1986).

$$\frac{dx}{dt} = kx(1-\gamma x) \tag{6}$$

The second order term in the equation was introduced to explain the lag observed in the mineralization of both the substrates (wood and atrazine), which could be attributed to the addition of *P. chrysosporium* in the form of spores rather than as mycelia. Integrating Equation 6 gives:

$$\int \frac{dx}{kx(1-\gamma x)} = \int dt \tag{7}$$

$$\Rightarrow \int \frac{dx}{kx} + \frac{\gamma}{k}\int \frac{dx}{1-\gamma x} = \int dt \tag{8}$$

$$\Rightarrow \frac{1}{k}\ln\frac{x}{x_0} - \frac{\gamma}{k}\frac{1}{\gamma}\ln\frac{1-\gamma x}{1-\gamma x_0} = kt \tag{9}$$

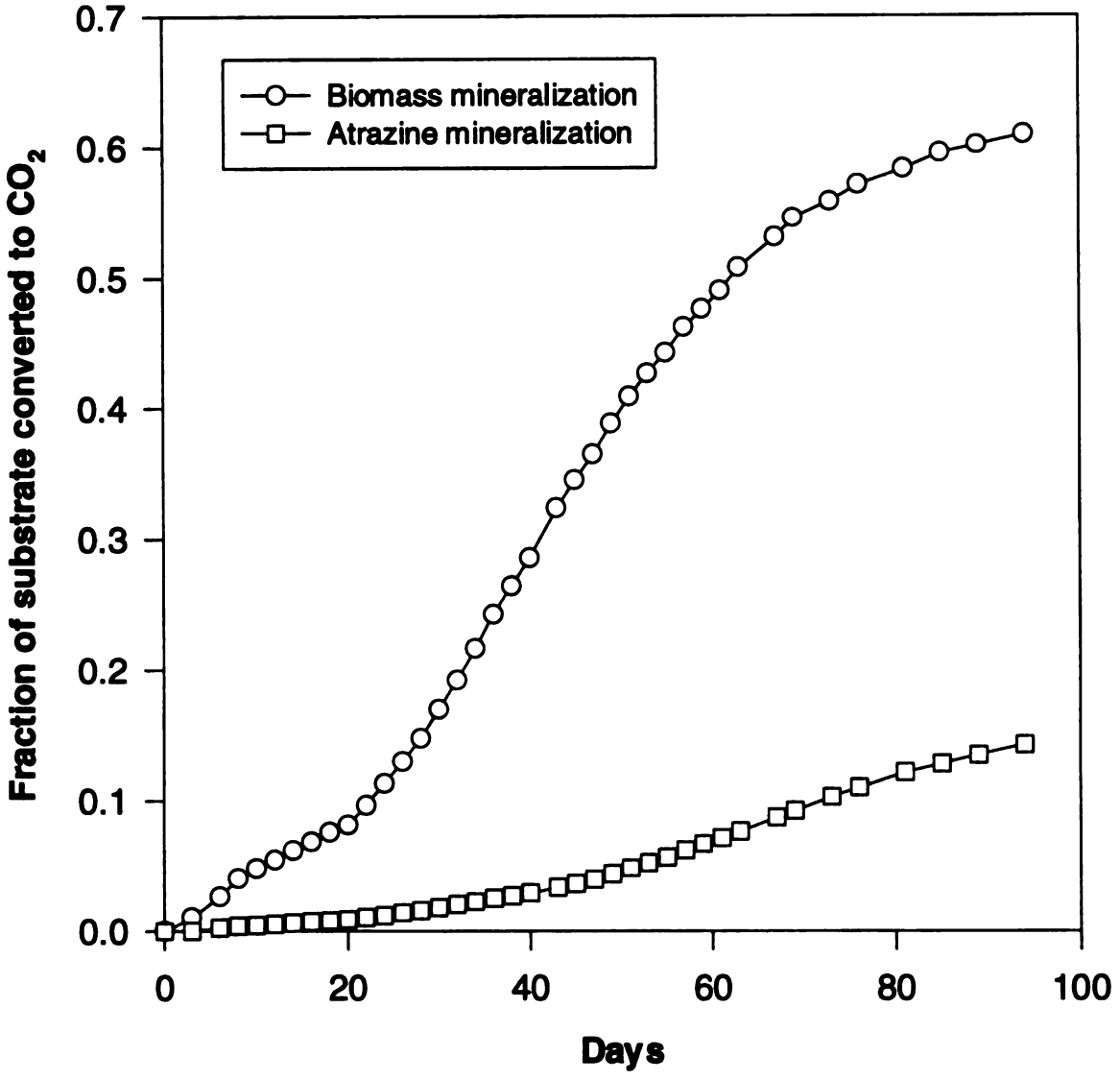

Figure 6.2 Mineralization of atrazine and wood during composting with an inoculum of *P. chrysosporium*.

$$\Rightarrow \ln\frac{x}{x_0} - \ln\frac{1-\gamma x}{1-\gamma x_0} = kt \tag{10}$$

$$\Rightarrow \frac{x}{x_0}\frac{1-\gamma x_0}{1-\gamma x} = e^{kt} \tag{11}$$

Upon rearranging this gives:

$$x = \frac{x_0 e^{kt}}{1-\gamma x_0 + \gamma x_0 e^{kt}} \tag{12}$$

This is the solution to Equation 6 and is a logistic curve that gives:

$$x_s = \frac{1}{\gamma} \tag{13}$$

where: x_s is the biomass at the end of the experiment.

The product formation kinetics (mineralization of wood and atrazine to CO_2) were modelled using the Ludeking-Piret equation (Bailey and Ollis, 1986) which combines growth associated and non-growth associated contributions.

$$\frac{dp}{dt} = \alpha\frac{dx}{dt} + \beta x \tag{14}$$

Integrating Equation 14 gives:

$$\int dp = \alpha\int dx + \beta\int x dt \tag{15}$$

Substituting the value of x from Equation 12 into Equation 14:

$$\Rightarrow p - p_o = \alpha(x - x_0) + \beta \int \frac{x_0 e^{kt} dt}{1 - \gamma x_0 + \gamma x_0 e^{kt}} \tag{16}$$

$$\Rightarrow p - p_0 = \alpha(x - x_0) + \frac{\beta}{\gamma k} \ln\left(1 - \gamma x_0 + \gamma x_0 e^{kt}\right) \tag{17}$$

Substituting Equations 12 and 13 in Equation 17:

$$\Rightarrow p - p_0 = \alpha x_0 \left(\frac{e^{kt}}{1 - \frac{x_0}{x_s} + \frac{x_0}{x_s} e^{kt}} - 1 \right) + \frac{\beta x_s}{k} \ln\left(1 - \frac{x_0}{x_s} + \frac{x_0}{x_s} e^{kt}\right) \tag{18}$$

Equation 18 was used to fit the experimental data obtained in Figure 6.2 using a commercially available software (PeakFit, Jandel Sientific). The results obtained from the process are as follows:

For biomass mineralization:

$$p - p_0 = (18.5)(0.1)\left(\frac{e^{0.085t}}{1 - 0.03 + 0.03e^{0.085t}} - 1 \right) \qquad (r^2 = 0.996) \tag{19}$$

For atrazine mineralization:

$$p - p_0 = (5.84)(0.1)\left(\frac{e^{0.05t}}{1 - 0.03 + 0.03e^{0.05t}} - 1 \right) \qquad (r^2 = 0.999) \tag{20}$$

From the correlation coefficients obtained we see that the theoretical model explains the experimental data very well. The model predictions for Equations 19 and 20 are shown in Figures 6.3 and 6.4 respectively. It is of interest to note that the model predicts all product formation to be growth associated with no non-growth associated contribution. The model also predicts a decrease in the lag with increase in the initial amount of biomass.

The preliminary process design and model predictions indicate the fesasibility of a large scale composting process for the disposal of pesticide-contaminated water.

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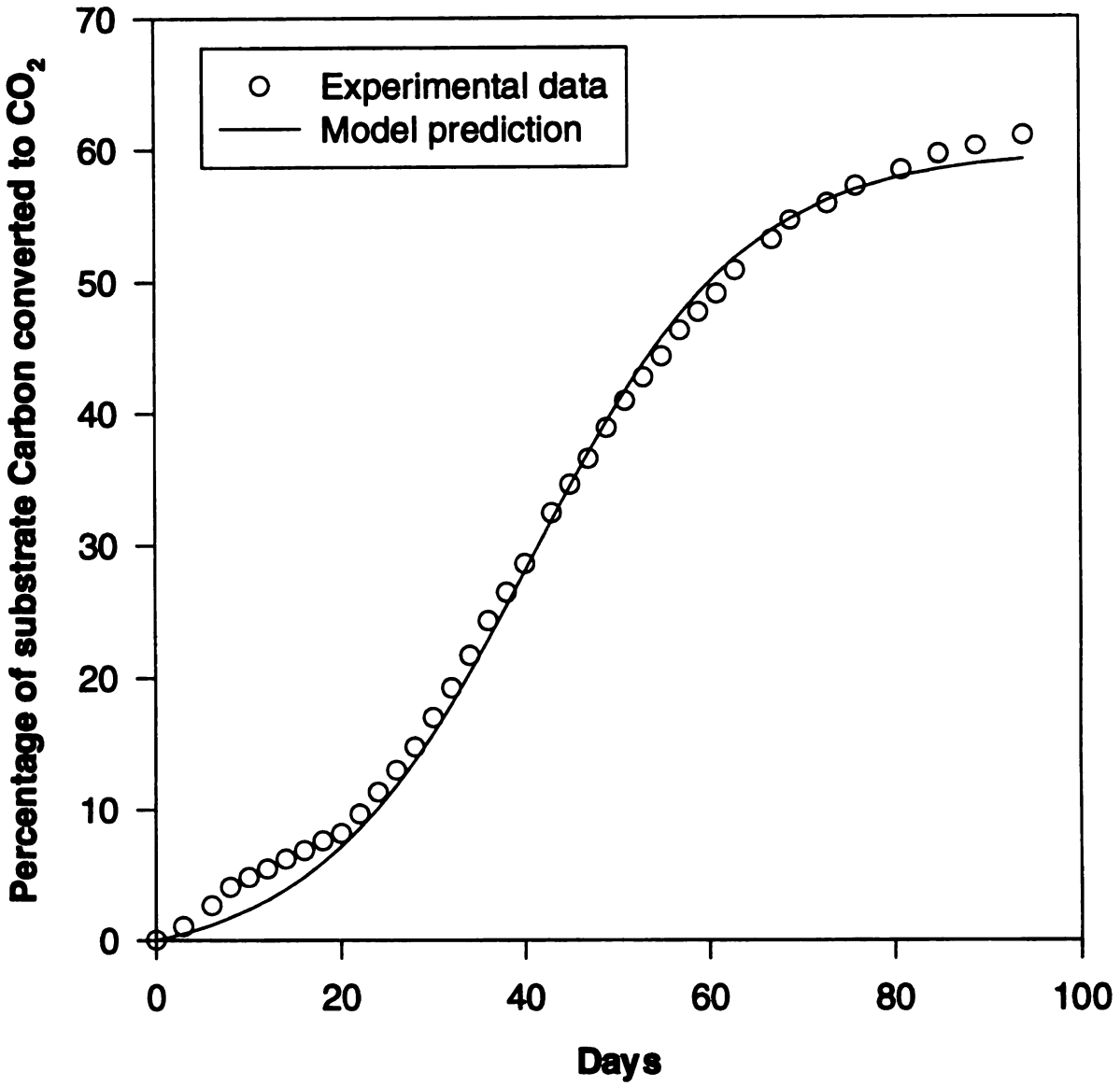

Figure 6.3 Model prediction for substrate (wood) mineralization.

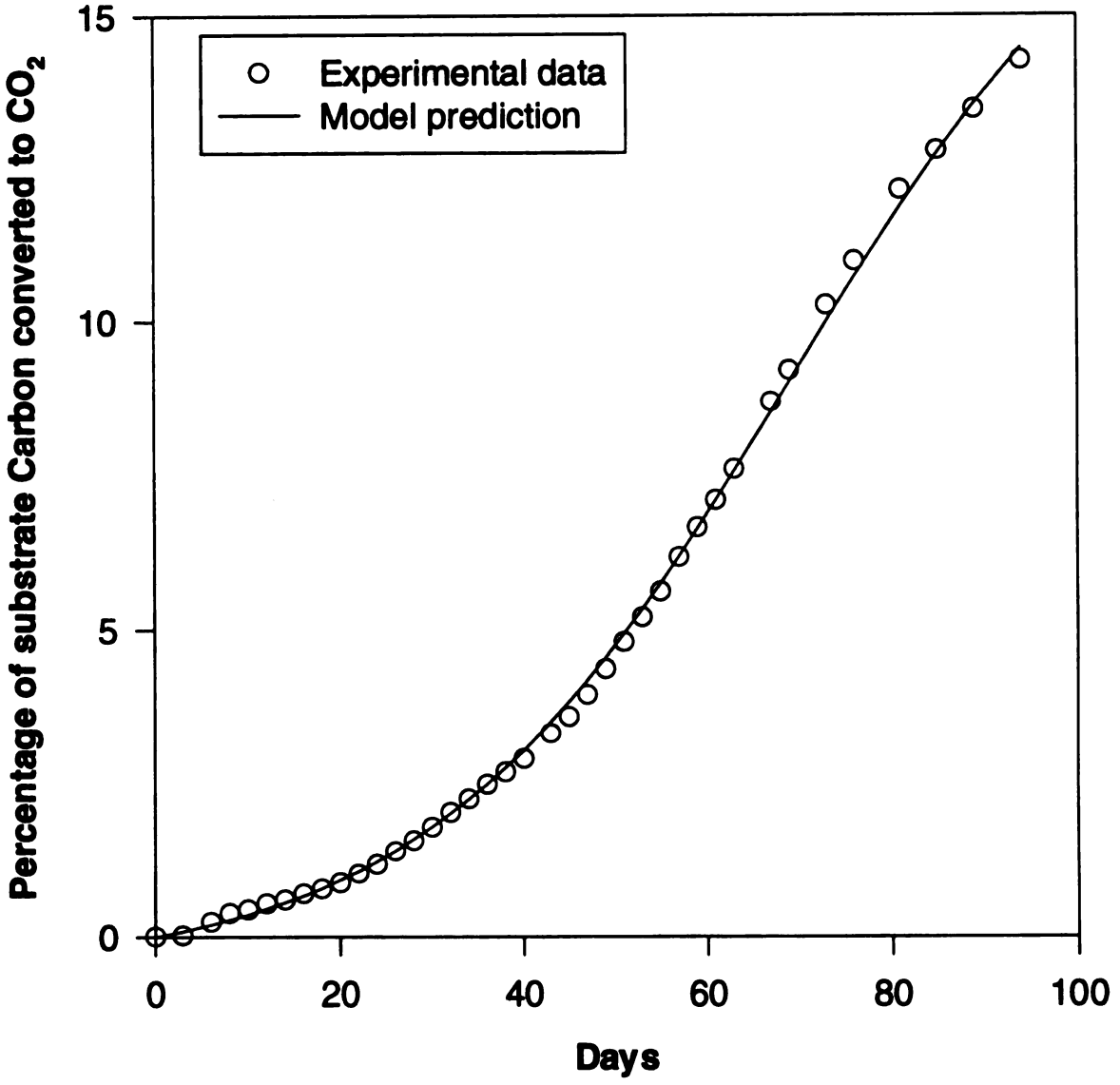

Figure 6.4 Model prediction for atrazine mineralization.

Chapter VII: Overall Conclusions and Future Directions

7.1 Overall Conclusions

The objective of this study was to investigate the potential for the degradation and mineralization of atrazine by gratuitous metabolism during the composting of lignocellulosic materials. Preliminary results indicated that a moisture content of 70% and higher C/N ratios resulted in a greater conversion of the poplar wood substrate to CO_2. Results from a study on the effect of temperature on the mineralization of poplar wood and atrazine indicated that substrate conversion during lab-scale composting was optimal at a mesophilic temperature of 37°C, though atrazine mineralization was minimal at all temperatures. Subsequent composting experiments conducted at 37°C with untreated and pretreated lignocellulosic substrates showed that pretreating the substrates had little effect on the mineralization of atrazine. Mineralization of atrazine was significantly enhanced when *P. chrysosporium*, a wood degrading white-rot fungus, was added as an inoculum to the composters. In summary, under the lab-scale composting conditions maintained in our study, optimum mineralization of atrazine was achieved at a moisture content of 70%, composting temperature of 37°C, and with the addition of an exogenous inoculum of *P. chrysosporium*.

This study represents a significant step forward in the field of composting as a disposal option for pesticide contaminated sources, since research has not been conducted previously to investigate the co-metabolism of pesticides during the composting of poplar wood. This is also the first direct evidence of atrazine mineralization during the composting of lignocellulosic substrates with *P. chrysosporium*. This seems to suggest the

atrazine by ring cleavage, since earlier investigators reported no mineralization of atrazine (by ring cleavage) by *P. chrysosporium* in pure cultures (Mougin et al., 1994) and in soils amended with *P. chrysosporium* (Hickey et al., 1994).

7.2 Future Directions

A pilot scale composting operation to confirm the results obtained in the laboratory scale experiments would be a logical extension to this study. The pilot scale operation would also confirm the results of the preliminary process design and modelling. An elucidation of the role of lignocellulosic materials in the mineralization of atrazine by *P. chrysosporium*, including a clarification of the enzymatic processes that lead to the mineralization of atrazine would be another area that could be explored. This could be carried out by using shake flask cultures of *P. chrysosporium* with atrazine and wood as substrates, since isolating the enzymes responsible for atrazine mineralization in a solid state fermentation environment might be difficult. Another area that can be investigated is the effect of different types of wood or lignocellulosics on the mineralization of atrazine by *P. chrysosporium*, based on the evidence that different types of wood have been shown to induce the expression of different enzymes and/or different levels of enzymes. Also, the effect of pesticide loading rates on pesticide mineralization is another area that bears investigation.

Appendix A

Effect of C/N Ratio and Moisture Content on the Composting of Poplar Wood

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SUMMARY

Milled poplar wood (1.7 mm mesh size) was composted in lab-scale reactors. Initial C/N ratios were adjusted to 10:1, 30:1, and 50:1 using urea as the nitrogen source. At each C/N ratio, three moisture levels (30, 50, and 70%) were tested. C/N ratios of 50:1 or 30:1 and moisture content of 70% favored more effective composting as indicated by higher levels of mineralization of the poplar wood to CO_2.

INTRODUCTION

Composting is fast becoming the primary disposal option for solid wastes such as leaves, grass, and woody materials, because of the proposed ban on the land filling and incineration of these wastes in many states in the U.S. Compared to the composting of yard wastes (Michel et al., 1993) there have been relatively few studies to date on the composting of woody materials such as wood pallets, wood processing residues, and Christmas trees. Furthermore, substantial variations in results were reported by different investigators. For example, Poincelot and Day (1973) showed that changing the C/N ratio from 41:1 to 35:1 nearly doubled the amount of degradation of leaf cellulose. Campbell and Tripepi (1991) recommended a relatively broad target C/N ratio of 40:1 to 70:1 for effective composting of wood wastes. Darbyshire et al. (1989) reported that a C/N ratio of 39:1 and a moisture content of 60% are effective for the decomposition of milled spruce bark, while Haug (1980) on the other hand indicated that high moisture content hinders

aeration and induces undesirable anaerobic conditions during composting. In this study, we used a laboratory-scale composting system recently designed by us (Michel et al., 1993) to investigate how the mineralization of milled poplar wood is affected in composters with varying moisture contents (30, 50, or 70%) and initial C/N ratios of 10:1, 30:1, and 50:1.

MATERIALS AND METHODS

Compost substrate. The poplar wood used in this study was a hybrid between *Populus nigra* and *Populus deltoides* and has been designated *Populus x euramericana* eugenei, and was grown at the Kellogg Biological Station and was provided by the NSF Center for Microbial Ecology at Michigan State University. The poplar wood was ground in a Wiley mill through a #10 screen (1.7 mm mesh size). The substrate was amended with urea to give C/N ratios of 10:1, 30:1, and 50:1, and enough sterile distilled water was added as a spray to give moisture contents of 30%, 50%, or 70%.

Compost inoculum. The inoculum used for composting was obtained from 10-week-old wood compost piles operated by a large scale composting facility (Hollandia Gardens, Holland, MI). A 10% w/w inoculum was used for all composters.

Composting System. Composting was carried out in a laboratory scale composting system described by Michel et al. (1993). In brief, the system consisted of rubber-stoppered 2-liter, wide mouth glass jars with two plastic screens (1cm and 1mm mesh opening) forming a false bottom. Aeration was provided through a hole just below

the level of the two screens. CO_2-free, humidified air for the composters was provided by passing the air through a flask containing 5 N NaOH to remove CO_2 and then through a 5 gallon carboy containing 2.5 gallons of distilled water. The air flow to each of the composters was set at 100 ml/min by means of a needle valve placed just upstream of the composters. The exhaust gas from each composter passed through two 5N NaOH containers to trap CO_2 present in the exhaust gas. The amount of CO_2 trapped was measured as described by Michel et al. (1993). The entire system was maintained at 37°C.

RESULTS AND DISCUSSION

Milled poplar was used in this study since reduction in particle size has been shown to increase the decomposition and humification rates during composting (N'Dayegamiye and Isfan, 1991; Poincelot, 1974). Mineralization of biomass as measured by the CO_2 released from the composters allowed comparison of the effect of moisture content and C/N ratio on the composting of poplar wood. The time course of organic matter mineralization at an initial C/N ratio of 10:1 and varying moisture content is shown in Figure A.1. Mineralization of biomass was rather minimal (<3%) at 30% and 50% moisture as compared to 15% mineralization observed at 70% moisture.

CO_2 production profiles from the composters with an initial C/N ratio of 30:1 and varying moisture contents are shown in Figure A.2. The extent of mineralization in composters with 30 and 50% moisture was roughly comparable in the initial stages and

was virtually identical at the end of 108 days. In comparison, the rate and extent of CO_2 generation was much greater at 70% moisture, with no detectable lag.

Mineralization trends in composters with initial C/N ratio of 50:1 (Figure A.3) were similar to those observed in composters with an initial C/N ratio of 30:1. For example, the extent of conversion of substrate carbon to CO_2 observed in composters with 30 and 50% moisture content was relatively low at C/N ratios of both 30:1 and 50:1. Also, the behavior of the composters with 70% moisture content and C/N ratios of 30:1 and 50:1 were nearly identical with about 15.8% conversion of biomass to CO_2 (Figure A.4). This suggests that wood composting is effective even at a relatively high C/N ratio of 50:1. The results presented in Figure A.4 also show that even the control run without the addition of nitrogen (C/N ratio of 820:1) showed mineralization of substrate carbon comparable to that observed in composters with C/N ratio of 30:1 and moisture contents of 30 and 50%.

The results clearly indicate that mineralization of the substrate occurs optimally with a C/N ratio of 30:1 to 50:1 and a moisture content of 70% and further suggest that lower C/N ratios tend to be inhibitory to composting. Our results are in agreement with those of Zadrazil (1980) who showed that low concentrations of nitrogen in the form of ammonium nitrate (0.25%) increased decomposition rates of straw while high levels of ammonium nitrate (0.75 and 1.25%) hindered decomposition.

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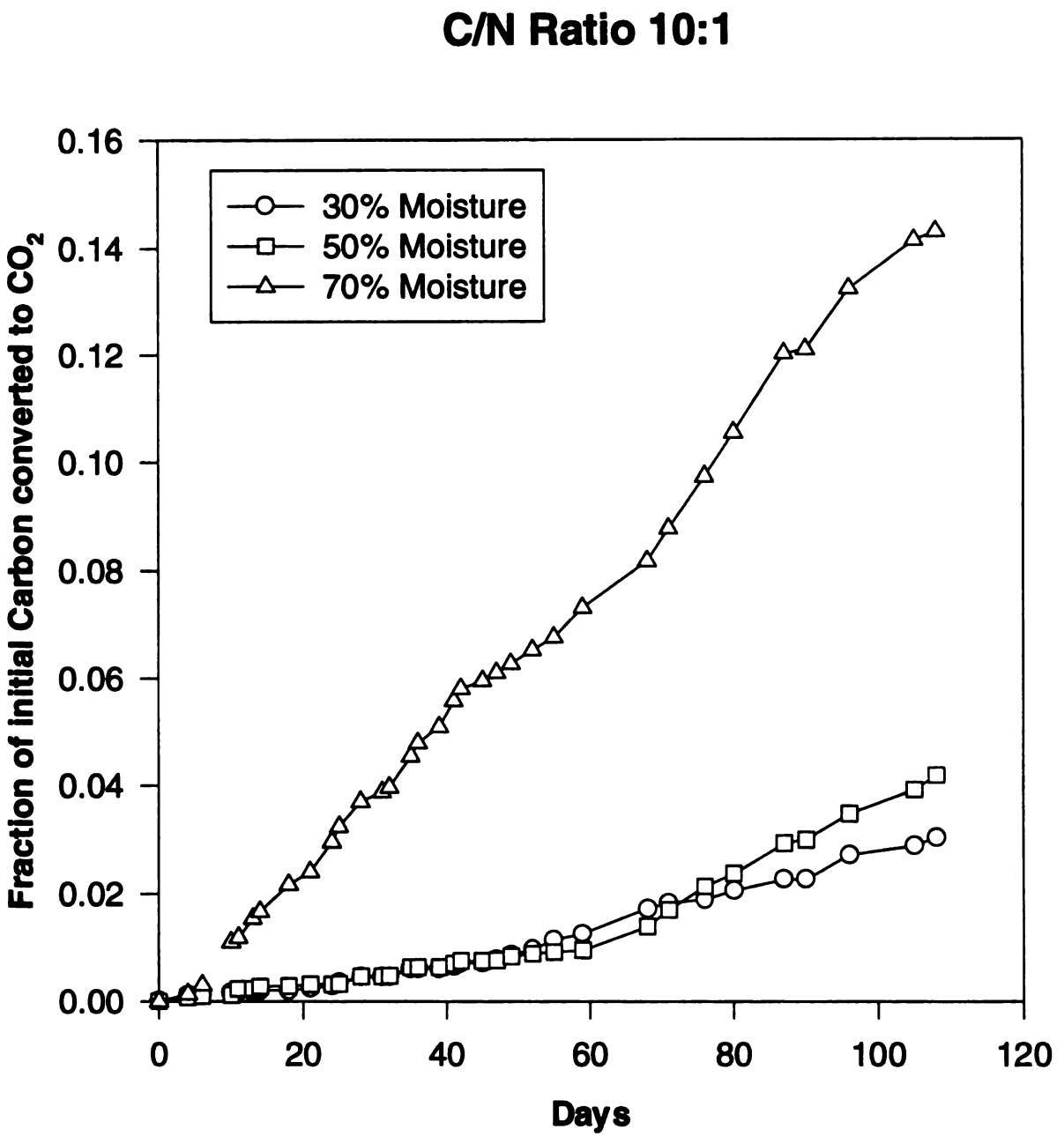

Figure A.1: Conversion of initial carbon to CO_2 during the composting of poplar wood at an initial C/N ratio of 10:1 and varying moisture contents.

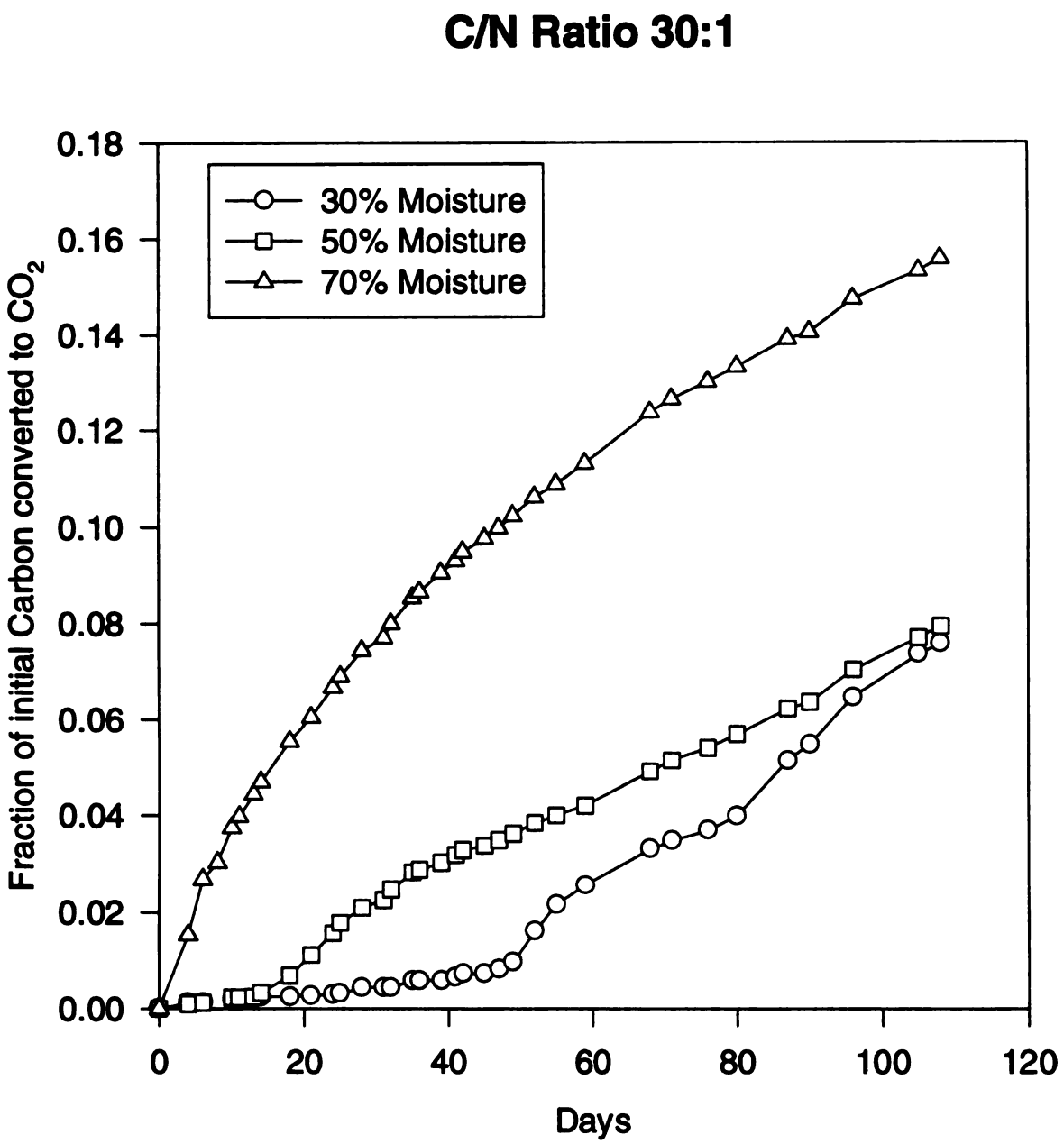

Figure A.2 Conversion of initial carbon to CO_2 during the composting of poplar wood at an initial C/N ratio of 30:1 and varying moisture contents.

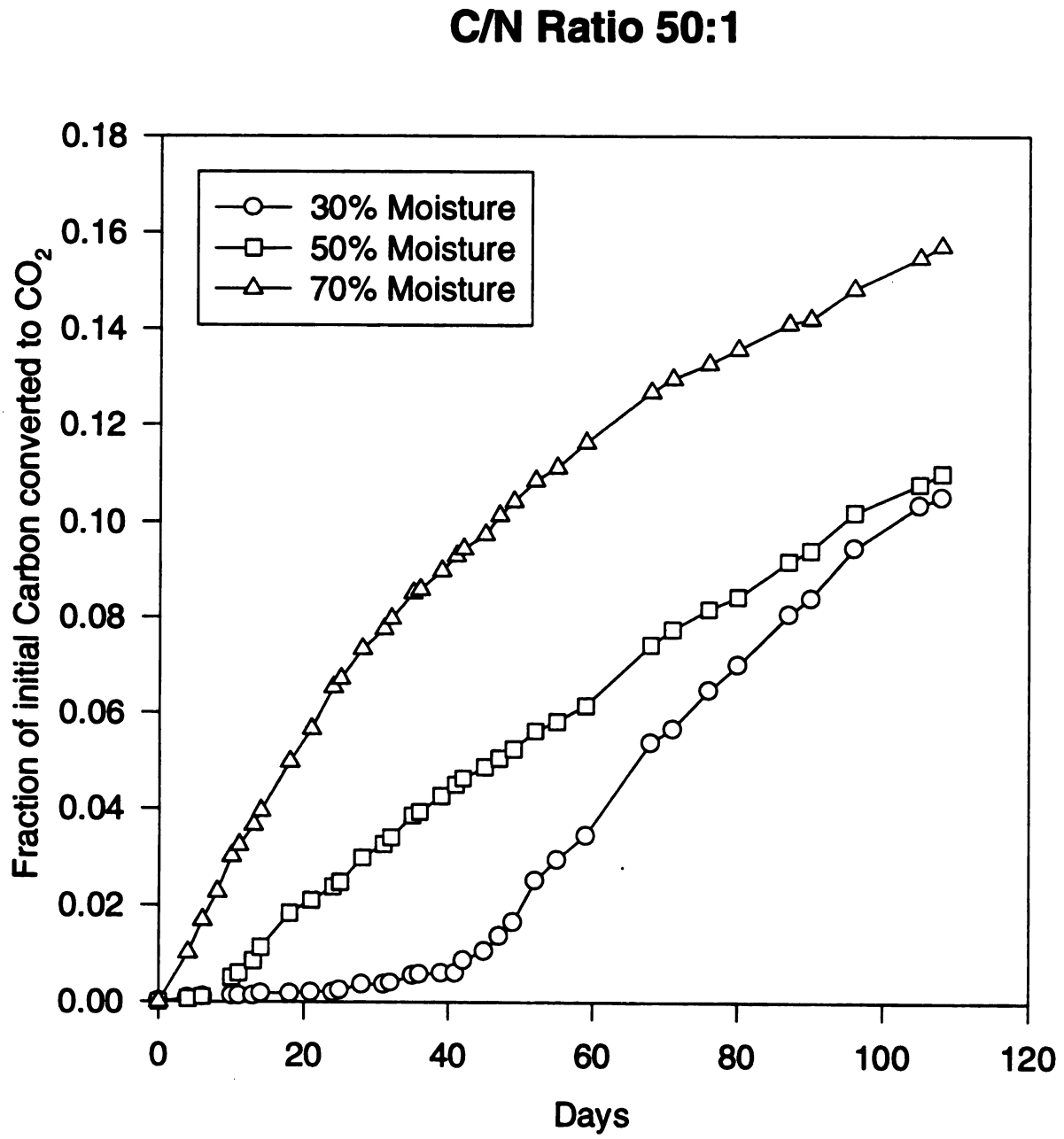

Figure A.3 Conversion of initial carbon to CO_2 during the composting of poplar wood at an initial C/N ratio of 50:1 and varying moisture contents.

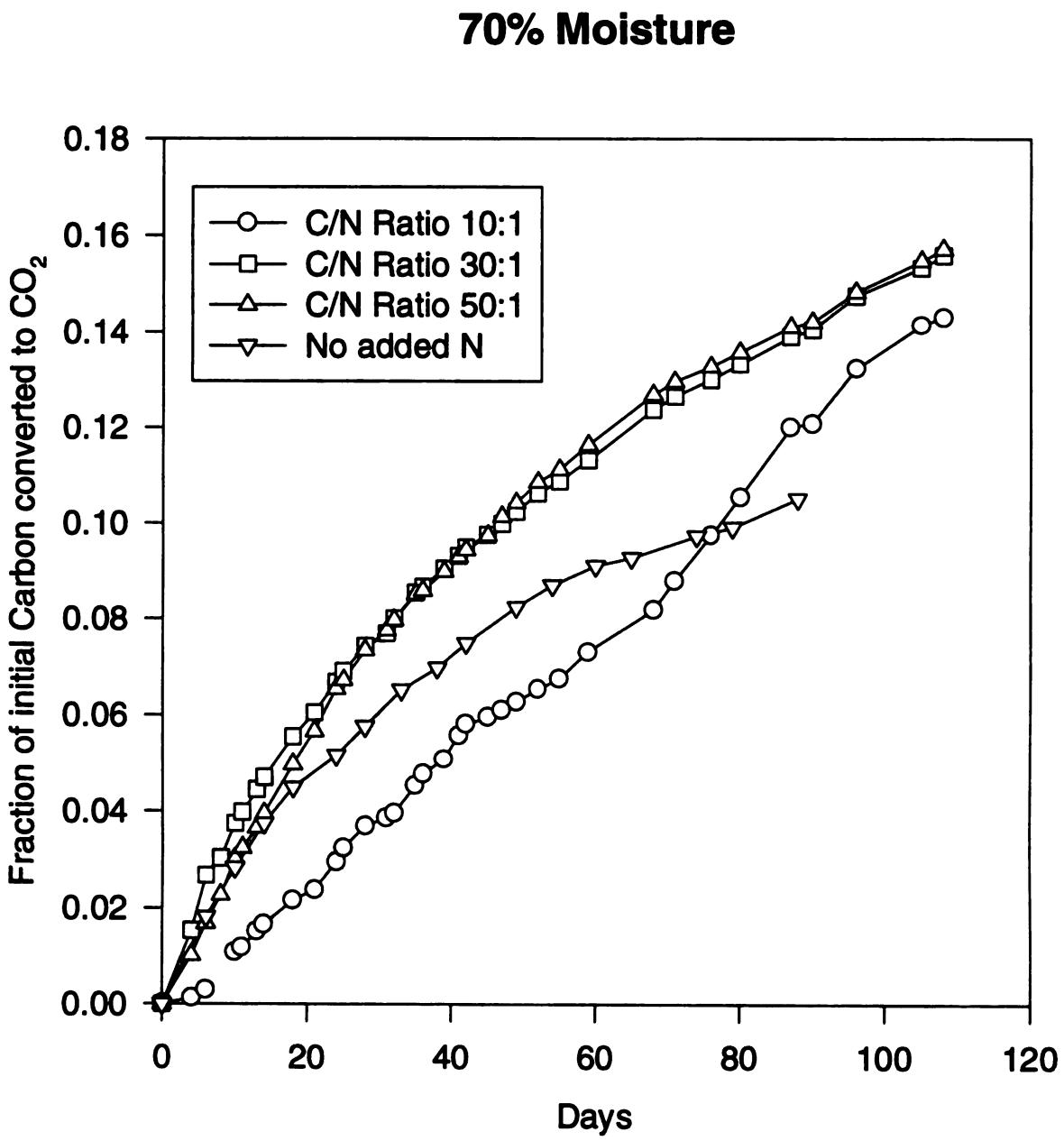

Figure A.4: Conversion of initial carbon to CO_2 during the composting of poplar wood at an initial moisture content of 70% and varying C/N ratios.

APPENDIX B

Tabulated Data

Table B.1: Data for Figure 3.1

Day	Wood 55°C Average	Wood 55°C Half range	Wood 25°C Average	Wood 25°C Half range	Day	Wood 37°C Average	Wood 37°C Half range
0	0.0000	0.0000	0.0000	0.0000	0	0.0000	0.0000
10	0.0110	0.0011	0.0099	0.0004	6	0.0182	0.0022
14	0.0153	0.0013	0.0143	0.0000	10	0.0284	0.0056
20	0.0210	0.0018	0.0169	0.0000	14	0.0375	0.0066
26	0.0269	0.0001	0.0210	0.0000	18	0.0450	0.0059
32	0.0307	0.0016	0.0241	0.0001	24	0.0516	0.0064
35	0.0338	0.0007	0.0261	0.0001	28	0.0576	0.0041
41	0.0369	0.0009	0.0299	0.0001	33	0.0651	0.0046
45	0.0416	0.0007	0.0324	0.0004	38	0.0697	0.0054
49	0.0422	0.0001	0.0334	0.0006	42	0.0749	0.0042
53	0.0453	0.0000	0.0352	0.0010	49	0.0824	0.0045
59	0.0476	0.0006	0.0376	0.0012	54	0.0870	0.0044
63	0.0501	0.0010	0.0397	0.0008	60	0.0911	0.0032
68	0.0532	0.0005	0.0431	0.0009	65	0.0927	0.0025
73	0.0550	0.0003	0.0439	0.0012	74	0.0973	0.0020
77	0.0573	0.0012	0.0452	0.0014	79	0.0992	0.0027
81	0.0577	0.0012	0.0461	0.0012	88	0.1051	0.0057
84	0.0590	0.0014	0.0465	0.0012			

Table B.2: Data for Figure 3.2

Day	Wood 55°C Average	Wood 55°C Half range	Wood 25°C Average	Wood 25°C Half range	Day	Wood 37°C Average	Wood 37°C Half range
0	0.0000	0.0000	0.0000	0.0000	0	0.0000	0.0000
10	0.1044	0.0174	0.0963	0.0093	6	0.1389	0.0365
14	0.1492	0.0146	0.1962	0.0035	10	0.3286	0.0333
20	0.2570	0.0031	0.3923	0.0068	14	0.4363	0.0331
26	0.4768	0.0241	0.5411	0.0656	18	0.4522	0.0423
32	0.7942	0.0772	0.7633	0.0958	24	0.5677	0.0665
35	0.8165	0.0583	0.8423	0.0905	28	0.5814	0.0792
41	0.8953	0.0471	0.9949	0.0912	33	0.6814	0.0646
45	0.9206	0.0550	0.9949	0.0912	38	0.7378	0.0661
49	0.9664	0.0666	1.0311	0.0926	42	0.7895	0.0639
53	0.9997	0.0468	1.1345	0.0649	49	0.8766	0.0509
59	1.0056	0.0410	1.1757	0.0718	54	0.9565	0.0486
63	1.0377	0.0568	1.1939	0.0821	60	1.0663	0.0544
68	1.0643	0.0478	1.2242	0.0772	65	1.1275	0.0709
73	1.1023	0.0602	1.2691	0.0872	74	1.2250	0.1610
77	1.1133	0.0712	1.2984	0.0752	79	1.3160	0.2428
81	1.1176	0.0756	1.3281	0.0855	88	1.4680	0.3719
84	1.1609	0.0753	1.3516	0.0754			

Table B.3: Data for Figure 3.3

	Wood 55°C		Cobs 55°C	
Day	Average	Half range	Average	Half range
0	0.0000	0.0000	0.0000	0.0000
10	0.0110	0.0011	0.0319	0.0002
14	0.0153	0.0013	0.0455	0.0009
20	0.0210	0.0018	0.0580	0.0008
26	0.0269	0.0001	0.0754	0.0010
32	0.0307	0.0016	0.0819	0.0005
35	0.0338	0.0007	0.0909	0.0002
41	0.0369	0.0009	0.1010	0.0045
45	0.0416	0.0007	0.1082	0.0075
49	0.0422	0.0001	0.1123	0.0095
53	0.0453	0.0000	0.1220	0.0132
59	0.0476	0.0006	0.1325	0.0111
63	0.0501	0.0010	0.1375	0.0107
68	0.0532	0.0005	0.1454	0.0099
73	0.0550	0.0003	0.1511	0.0095
77	0.0573	0.0012	0.1544	0.0096
81	0.0577	0.0012	0.1547	0.0099
84	0.0590	0.0014	0.1591	0.0114

Table B.4: Data for Figure 3.4

	Wood 55°C		Cobs 55°C	
Day	Average	Half range	Average	Half range
0	0.0000	0.0000	0.0000	0.0000
10	0.1044	0.0174	0.2843	0.0159
14	0.1492	0.0146	0.3077	0.0191
20	0.2570	0.0031	0.3284	0.0265
26	0.4768	0.0241	0.3682	0.0421
32	0.7942	0.0772	0.3871	0.0330
35	0.8165	0.0583	0.3950	0.0410
41	0.8953	0.0471	0.4197	0.0348
45	0.9206	0.0550	0.4263	0.0394
49	0.9664	0.0666	0.4453	0.0356
53	0.9997	0.0468	0.4570	0.0279
59	1.0056	0.0410	0.4897	0.0291
63	1.0377	0.0568	0.5094	0.0313
68	1.0643	0.0478	0.5325	0.0347
73	1.1023	0.0602	0.5799	0.0449
77	1.1133	0.0712	0.5824	0.0424
81	1.1176	0.0756	0.5962	0.0286
84	1.1609	0.0753	0.6213	0.0356

Table B.5: Data for Figure 3.5

		$ChCl_3$	MeOH	NaOH	H_2O	CO_2	Bound
Wood 25°C	Day 0	100491.3	4021.4	36.75	191.5	0	1090.059
	Day 30	8732782	3246580	1565572	188070.5	134334.5	1645452
	Day 60	7623253	2782227	1505333	229962.1	206916	2350058
Wood 37°C	Day 0	100491.3	4021.4	36.75	191.5	0	1090.059
	Day 30	2977954	7031470	2895231	229583.6	151383.5	2583450
	Day 60	1399850	7701231	3062916	159840.3	451659.5	2671062
Wood 55°C	Day 0	100491.3	4021.4	36.75	191.5	0	1090.059
	Day 30	2196903	6563652	2176271	325801.4	139774.5	3681661
	Day 60	2038878	6009132	2182022	258163.5	176984	4229462
Cobs 55°C	Day 0	114153	2784.4	737.75	74.2	0	1225.44
	Day 30	1375303	4191373	9444908	377663.1	68125	1214582
	Day 60	1298248	3785900	8526447	373885	86179	1315579

Table B.6: Data for Figure 4.1

	Native		STEX		AFEX		Newspaper	
Hours	Average	Std.Dev.	Average	Std.Dev.	Average	Std.Dev.	Average	Std.Dev.
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	7.192	0.114	28.422	0.231	16.096	0.255	16.240	0.187
1	8.219	0.000	38.358	0.231	18.380	0.471	21.958	0.323
2	9.818	0.000	51.760	0.462	22.718	0.731	26.914	0.656
4	11.873	0.000	67.126	2.195	28.768	0.666	33.166	0.494
8	15.069	0.000	78.448	1.502	35.218	0.797	38.275	0.755
12	15.412	0.114	80.990	1.733	37.844	0.987	41.401	0.988
24	19.864	0.000	90.117	3.004	46.349	0.323	46.204	1.347
48	21.804	0.342	91.504	3.466	51.524	0.215	48.339	1.029

Table B.7: Data for Figure 4.2

	Native		AFEX		STEX		Paper	
Day	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.0182	0.0022	0.0735	0.0158	0.0043	0.0008	0.0076	0.0014
10	0.0284	0.0056	0.0931	0.0092	0.0059	0.0009	0.0110	0.0015
14	0.0375	0.0066	0.1155	0.0059	0.0073	0.0011	0.0146	0.0023
18	0.0450	0.0059	0.1329	0.0035	0.0077	0.0012	0.0152	0.0023
24	0.0516	0.0064	0.1480	0.0080	0.0097	0.0025	0.0187	0.0025
28	0.0576	0.0041	0.1699	0.0107	0.0118	0.0032	0.0216	0.0019
33	0.0651	0.0046	0.2045	0.0272	0.0141	0.0036	0.0239	0.0020
38	0.0697	0.0054	0.2484	0.0267	0.0157	0.0050	0.0269	0.0020
42	0.0749	0.0042	0.2870	0.0404	0.0185	0.0059	0.0353	0.0078
49	0.0824	0.0045	0.3248	0.0493	0.0250	0.0108	0.0553	0.0284
54	0.0870	0.0044	0.3484	0.0547	0.0390	0.0154	0.0734	0.0491
60	0.0911	0.0032	0.3672	0.0575	0.0495	0.0190	0.0881	0.0672
65	0.0927	0.0025	0.3791	0.0556	0.0534	0.0194	0.0994	0.0783
74	0.0973	0.0020	0.4033	0.0552	0.0723	0.0238	0.1085	0.0840
79	0.0992	0.0027	0.4129	0.0559	0.0829	0.0217	0.1108	0.0853
88	0.1051	0.0057	0.4388	0.0607	0.1009	0.0217	0.1233	0.0965
95	0.1079	0.0055	0.4538	0.0631	0.1087	0.0236	0.1357	0.1123
102	0.1119	0.0048	0.4654	0.0647	0.1167	0.0238	0.1461	0.1240
108	0.1159	0.0051	0.4768	0.0680	0.1218	0.0242	0.1574	0.1352
112	0.1220	0.0046	0.4867	0.0703	0.1268	0.0244	0.1629	0.1374
124	0.1279	0.0045	0.5067	0.0754	0.1364	0.0247	0.1799	0.1569
130	0.1301	0.0053	0.5197	0.0786	0.1423	0.0232	0.1868	0.1641
138	0.1358	0.0044	0.5303	0.0781	0.1503	0.0198	0.1928	0.1676
143	0.1363	0.0041	0.5338	0.0781	0.1521	0.0198	0.1971	0.1717
151	0.1406	0.0048	0.5437	0.0809	0.1557	0.0201	0.2032	0.1769
160	0.1474	0.0051	0.5574	0.0844	0.1642	0.0173	0.2102	0.1805

Table B.8: Data for Figure 4.3

Day	Native		AFEX		STEX		Paper	
	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.1389	0.0365	0.1957	0.0078	0.0194	0.0031	0.4755	0.0008
10	0.3286	0.0333	0.2654	0.0302	0.0305	0.0045	0.6437	0.0202
14	0.4363	0.0331	0.2893	0.0253	0.0367	0.0060	0.7737	0.0260
18	0.4522	0.0423	0.2923	0.0279	0.0547	0.0005	0.8646	0.0102
24	0.5677	0.0665	0.3296	0.0114	0.0691	0.0047	0.9671	0.0212
28	0.5814	0.0792	0.3490	0.0088	0.0828	0.0063	1.1497	0.0373
33	0.6814	0.0646	0.3566	0.0152	0.1029	0.0060	1.2381	0.0325
38	0.7378	0.0661	0.3566	0.0152	0.1094	0.0031	1.2530	0.0475
42	0.7895	0.0639	0.3579	0.0140	0.1415	0.0316	1.3493	0.0775
49	0.8766	0.0509	0.4134	0.0027	0.1558	0.0173	1.4846	0.1354
54	0.9565	0.0486	0.4731	0.0234	0.1897	0.0140	1.6385	0.2285
60	1.0663	0.0544	0.5921	0.0751	0.2367	0.0056	1.6886	0.2131
65	1.1275	0.0709	0.7018	0.1848	0.2637	0.0049	1.7074	0.1958
74	1.2250	0.1610	0.9104	0.2358	0.8429	0.0806	1.8623	0.2515
79	1.3160	0.2428	0.9823	0.2200	0.9552	0.0382	1.9123	0.2640
88	1.4680	0.3719	1.2872	0.3573	1.0261	0.0042	2.2399	0.5024
95	1.7074	0.3780	1.6159	0.4024	1.2272	0.0504	2.6979	0.9117
102	1.8083	0.3399	1.7900	0.3881	1.2363	0.0510	3.0564	1.2171
108	1.8906	0.2645	2.0031	0.4560	1.3220	0.0152	3.3931	1.5035
112	2.0476	0.3268	2.1164	0.4173	1.3627	0.0412	3.7414	1.7806
124	2.2618	0.2577	2.5502	0.4315	1.4571	0.0574	4.3262	2.2565
130	2.4391	0.1949	2.7931	0.5249	1.4975	0.0691	5.0510	2.8466
138	2.9131	0.0650	3.6687	0.6052	1.6441	0.0912	5.4196	2.9923
143	2.9424	0.0878	3.7422	0.5904	1.6627	0.0899	5.8632	3.3973
151	3.0985	0.0018	4.0361	0.5345	1.7368	0.0791	6.5909	3.9778
160	3.3014	0.0745	4.3016	0.4518	1.7736	0.0968	6.9527	4.3037

Table B.9: Data for Figure 4.4

Day	Native		AFEX		STEX		Paper	
	Total CO_2	$^{14}CO_2$	Total CO_2	$^{14}CO_2$	Total CO_2	$^{14}CO_2$	Total CO_2	$^{14}CO_2$
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.0182	0.0139	0.0735	0.0196	0.0043	0.0019	0.0076	0.0476
10	0.0284	0.0329	0.0931	0.0265	0.0059	0.0031	0.0110	0.0644
14	0.0375	0.0436	0.1155	0.0289	0.0073	0.0037	0.0146	0.0774
18	0.0450	0.0452	0.1329	0.0292	0.0077	0.0055	0.0152	0.0865
24	0.0516	0.0568	0.1480	0.0330	0.0097	0.0069	0.0187	0.0967
28	0.0576	0.0581	0.1699	0.0349	0.0118	0.0083	0.0216	0.1150
33	0.0651	0.0681	0.2045	0.0357	0.0141	0.0103	0.0239	0.1238
38	0.0697	0.0738	0.2484	0.0357	0.0157	0.0109	0.0269	0.1253
42	0.0749	0.0790	0.2870	0.0358	0.0185	0.0142	0.0353	0.1349
49	0.0824	0.0877	0.3248	0.0413	0.0250	0.0156	0.0553	0.1485
54	0.0870	0.0957	0.3484	0.0473	0.0390	0.0190	0.0734	0.1638
60	0.0911	0.1066	0.3672	0.0592	0.0495	0.0237	0.0881	0.1689
65	0.0927	0.1127	0.3791	0.0702	0.0534	0.0264	0.0994	0.1707
74	0.0973	0.1225	0.4033	0.0910	0.0723	0.0843	0.1085	0.1862
79	0.0992	0.1316	0.4129	0.0982	0.0829	0.0955	0.1108	0.1912
88	0.1051	0.1468	0.4388	0.1287	0.1009	0.1026	0.1233	0.2240
95	0.1079	0.1707	0.4538	0.1616	0.1087	0.1227	0.1357	0.2698
102	0.1119	0.1808	0.4654	0.1790	0.1167	0.1236	0.1461	0.3056
108	0.1159	0.1891	0.4768	0.2003	0.1218	0.1322	0.1574	0.3393
112	0.1220	0.2048	0.4867	0.2116	0.1268	0.1363	0.1629	0.3741
124	0.1279	0.2262	0.5067	0.2550	0.1364	0.1457	0.1799	0.4326
130	0.1301	0.2439	0.5197	0.2793	0.1423	0.1497	0.1868	0.5051
138	0.1358	0.2913	0.5303	0.3669	0.1503	0.1644	0.1928	0.5420
143	0.1363	0.2942	0.5338	0.3742	0.1521	0.1663	0.1971	0.5863
151	0.1406	0.3098	0.5437	0.4036	0.1557	0.1737	0.2032	0.6591
160	0.1474	0.3301	0.5574	0.4302	0.1642	0.1774	0.2102	0.6953

Table B.10: Data for Figure 4.5

		$ChCl_3$	MeOH	NaOH	H_2O	CO_2	Bound
Native	Day 0	100491.3	4021.4	36.75	191.5	0	1090.059
	Day 40	1331614	1937492	1784633	59265.5	91639.0	3142859
	Day 160	134965.1	2427741	2261819	324811.3	410071.4	3551869
AFEX	Day 0	114153	2784.4	737.75	74.2	0	2920.203
	Day 40	212343.9	2183782	3097970	77457.6	44299.2	2948196
	Day 160	32787.68	1007718	4124086	458923.8	534306.2	3962125
STEX	Day 0	125399.6	5717.5	423.5	100	0	2262.97
	Day 40	1225898	4390178	2701964	65810.1	13588.05	1378757
	Day 160	79210.48	4110693	3514895	283612.2	220307.4	1700660
Paper	Day 0	195940.1	3583	415.25	311.6	0	4211.914
	Day 40	240017.8	1935721	2515004	32041.1	84537.5	3839674
	Day 160	80540.05	1588553	1838069	295513	413118.6	4576381

Table B.11: Data for Figure 5.1

Wood			Wood+PC		
Day	Average	Half range	Day	Average	Half range
0	0.0000	0.0000	0	0.0000	0.0000
6	0.0182	0.0022	3	0.0102	0.0017
10	0.0284	0.0056	6	0.0260	0.0026
14	0.0375	0.0066	8	0.0401	0.0011
18	0.0450	0.0059	10	0.0473	0.0004
24	0.0516	0.0064	12	0.0544	0.0024
28	0.0576	0.0041	14	0.0611	0.0046
33	0.0651	0.0046	16	0.0682	0.0058
38	0.0697	0.0054	18	0.0757	0.0098
42	0.0749	0.0042	20	0.0809	0.0117
49	0.0824	0.0045	22	0.0965	0.0183
54	0.0870	0.0044	24	0.1128	0.0262
60	0.0911	0.0032	26	0.1297	0.0323
65	0.0927	0.0025	28	0.1473	0.0353
74	0.0973	0.0020	30	0.1700	0.0362
79	0.0992	0.0027	32	0.1924	0.0359
88	0.1051	0.0057	34	0.2161	0.0356
95	0.1079	0.0055	36	0.2430	0.0344
			38	0.2640	0.0336
			40	0.2855	0.0343
			43	0.3241	0.0318
			45	0.3453	0.0311
			47	0.3651	0.0323
			49	0.3881	0.0292
			51	0.4088	0.0256
			53	0.4269	0.0212
			55	0.4427	0.0208
			57	0.4621	0.0155
			59	0.4757	0.0106
			61	0.4903	0.0064
			63	0.5077	0.0035
			67	0.5311	0.0071
			69	0.5455	0.0121
			73	0.5585	0.0205
			76	0.5708	0.0266
			81	0.5832	0.0316
			85	0.5956	0.0354
			89	0.6016	0.0393
			94	0.6095	0.0411

Table B.12: Data for Figure 5.2

Wood			Wood + PC		
Day	Average	Half range	Day	Average	Half range
0	0.0000	0.0000	0	0.0000	0.0000
16	0.0387	0.0001	8	0.0284	0.0003
24	0.0478	0.0035	16	0.0377	0.0003
31	0.0632	0.0088	23	0.0509	0.0016
33	0.0681	0.0114	25	0.0561	0.0026
40	0.0974	0.0200	32	0.0855	0.0086
49	0.1051	0.0240	41	0.1065	0.0189
56	0.1221	0.0265	48	0.1382	0.0107
59	0.1261	0.0273	51	0.1468	0.0089
64	0.1406	0.0222	56	0.1604	0.0077
68	0.1458	0.0258	60	0.1695	0.0064
76	0.1596	0.0286	68	0.1876	0.0057
83	0.1700	0.0314	75	0.2025	0.0049
			82	0.2222	0.0044

Table B.13: Data for Figure 5.3

Day	Wood + PC	
	Average	Half range
0	0.0000	0.0000
3	0.0142	0.0076
6	0.2417	0.0135
8	0.3719	0.0330
10	0.4430	0.0328
12	0.5364	0.0370
14	0.6024	0.0324
16	0.7054	0.0382
18	0.7835	0.0457
20	0.8762	0.0700
22	1.0268	0.1326
24	1.1825	0.2028
26	1.3864	0.2711
28	1.5496	0.3164
30	1.7862	0.3712
32	2.0093	0.4109
34	2.2396	0.4774
36	2.4821	0.5681
38	2.6765	0.6305
40	2.8923	0.7181
43	3.3072	0.8414
45	3.5863	0.9480
47	3.9340	1.1143
49	4.3576	1.1970
51	4.7891	1.3415
53	5.1962	1.4619
55	5.6148	1.6369
57	6.1718	1.7890
59	6.6537	1.8758
61	7.1023	1.9403
63	7.6064	1.9169
67	8.6895	1.9257
69	9.1997	1.8705
73	10.2577	1.6905
76	10.9807	1.5117
81	12.1437	1.1134
85	12.7868	0.8258
89	13.4585	0.5679
94	14.2570	0.3134

Day	Wood	
	Average	Half range
0	0.0000	0.0000
6	0.1389	0.0365
10	0.3286	0.0333
14	0.4363	0.0331
18	0.4522	0.0423
24	0.5677	0.0665
28	0.5814	0.0792
33	0.6814	0.0646
38	0.7378	0.0661
42	0.7895	0.0639
49	0.8766	0.0509
54	0.9565	0.0486
60	1.0663	0.0544
65	1.1275	0.0709
74	1.2250	0.1610
79	1.3160	0.2428
88	1.4680	0.3719

Table B.14: Data for Figure 5.4

	Wood+Corn		Wood+Corn+PC	
Day	Average	Half range	Average	Half range
0	0.0000	0.0000	0.0000	0.0000
3	0.0455	0.0012	0.0507	0.0010
6	0.0836	0.0068	0.1013	0.0004
8	0.1136	0.0092	0.1412	0.0111
10	0.1452	0.0042	0.1603	0.0201
12	0.1919	0.0010	0.2026	0.0213
14	0.2374	0.0001	0.2376	0.0104
16	0.2813	0.0021	0.2694	0.0025
18	0.3192	0.0027	0.2927	0.0105
20	0.3414	0.0025	0.3064	0.0155
22	0.3656	0.0060	0.3212	0.0200
24	0.3849	0.0099	0.3331	0.0215
26	0.4024	0.0116	0.3444	0.0251
28	0.4195	0.0137	0.3542	0.0262
30	0.4352	0.0157	0.3638	0.0284
32	0.4492	0.0172	0.3709	0.0303
34	0.4599	0.0161	0.3791	0.0317
36	0.4729	0.0178	0.3853	0.0327
38	0.4816	0.0183	0.3929	0.0349
40	0.4928	0.0187	0.4066	0.0332
43	0.5103	0.0203	0.4286	0.0291
45	0.5156	0.0206	0.4362	0.0270
47	0.5231	0.0206	0.4466	0.0239
49	0.5328	0.0217	0.4581	0.0221
51	0.5396	0.0219	0.4663	0.0203
53	0.5454	0.0228	0.4733	0.0181
55	0.5544	0.0225	0.4808	0.0195
57	0.5604	0.0225	0.4883	0.0184
59	0.5651	0.0227	0.4932	0.0176
61	0.5702	0.0233	0.4993	0.0169
63	0.5781	0.0232	0.5062	0.0159
67	0.5907	0.0232	0.5177	0.0142
69	0.5990	0.0230	0.5270	0.0139
73	0.6029	0.0233	0.5345	0.0115
76	0.6099	0.0223	0.5418	0.0116
81	0.6219	0.0217	0.5531	0.0114
85	0.6316	0.0201	0.5576	0.0113
89	0.6407	0.0169	0.5640	0.0117
94	0.6452	0.0124	0.5739	0.0135

	Wood	
Day	Average	Half range
0	0.0000	0.0000
6	0.0182	0.0022
10	0.0284	0.0056
14	0.0375	0.0066
18	0.0450	0.0059
24	0.0516	0.0064
28	0.0576	0.0041
33	0.0651	0.0046
38	0.0697	0.0054
42	0.0749	0.0042
49	0.0824	0.0045
54	0.0870	0.0044
60	0.0911	0.0032
65	0.0927	0.0025
74	0.0973	0.0020
79	0.0992	0.0027
88	0.1051	0.0057
95	0.1079	0.0055

Table B.15: Data for Figure 5.5

	Wood+Corn		Wood+Corn+PC	
DAY	Average	Half range	Average	Half range
0	0.0000	0.0000	0.0000	0.0000
3	0.2228	0.0155	0.2962	0.0507
6	0.2423	0.0146	0.3405	0.0689
8	0.3030	0.0271	0.3525	0.0642
10	0.3731	0.0448	0.3757	0.0441
12	0.4330	0.0640	0.4145	0.0210
14	0.4809	0.0636	0.5012	0.0470
16	0.5336	0.0653	0.5716	0.0612
18	0.5658	0.0626	0.6123	0.0525
20	0.5900	0.0617	0.6567	0.0664
22	0.6307	0.0361	0.7038	0.0862
24	0.6914	0.0009	0.7496	0.1148
26	0.7641	0.0519	0.7699	0.1236
28	0.8463	0.0985	0.7946	0.1278
30	0.9548	0.1669	0.8139	0.1325
32	1.0763	0.2402	0.8288	0.1403
34	1.2107	0.3309	0.8602	0.1386
36	1.4433	0.4435	0.8941	0.1479
38	1.6154	0.5289	0.9068	0.1523
40	1.7683	0.6159	0.9320	0.1491
43	2.0175	0.7370	1.1314	0.0032
45	2.1800	0.8447	1.2638	0.0805
47	2.3653	0.9274	1.4045	0.1474
49	2.5508	1.0268	1.5374	0.1847
51	2.7305	1.1214	1.6588	0.2146
53	2.8708	1.1918	1.7610	0.2386
55	3.1220	1.3562	1.8793	0.2514
57	3.2823	1.4160	2.0710	0.3487
59	3.4052	1.4704	2.1689	0.3642
61	3.5482	1.5315	2.2768	0.3933
63	3.6696	1.5722	2.3944	0.4223
67	3.9290	1.6965	2.6476	0.5067
69	4.0377	1.7288	2.7467	0.5408
73	4.2441	1.8218	2.9491	0.6247
76	4.4320	1.8451	3.1005	0.6828
81	4.6749	1.9067	3.3074	0.7345
85	4.9641	1.8413	3.4619	0.7883
89	5.1142	1.8425	3.6190	0.8457
94	5.2146	1.7670	3.8012	0.8844

	Wood	
Day	Average	Half range
0	0.0000	0.0000
6	0.1389	0.0365
10	0.3286	0.0333
14	0.4363	0.0331
18	0.4522	0.0423
24	0.5677	0.0665
28	0.5814	0.0792
33	0.6814	0.0646
38	0.7378	0.0661
42	0.7895	0.0639
49	0.8766	0.0509
54	0.9565	0.0486
60	1.0663	0.0544
65	1.1275	0.0709
74	1.2250	0.1610
79	1.3160	0.2428
88	1.4680	0.3719

Table B.16: Data for Figure 6.2

	Wood+PC	
Day	Total CO_2	$^{14}CO_2$
0	0.0000	0.0000
3	0.0102	0.0001
6	0.0260	0.0024
8	0.0401	0.0037
10	0.0473	0.0044
12	0.0544	0.0054
14	0.0611	0.0060
16	0.0682	0.0071
18	0.0757	0.0078
20	0.0809	0.0088
22	0.0965	0.0103
24	0.1128	0.0118
26	0.1297	0.0139
28	0.1473	0.0155
30	0.1700	0.0179
32	0.1924	0.0201
34	0.2161	0.0224
36	0.2430	0.0248
38	0.2640	0.0268
40	0.2855	0.0289
43	0.3241	0.0331
45	0.3453	0.0359
47	0.3651	0.0393
49	0.3881	0.0436
51	0.4088	0.0479
53	0.4269	0.0520
55	0.4427	0.0561
57	0.4621	0.0617
59	0.4757	0.0665
61	0.4903	0.0710
63	0.5077	0.0761
67	0.5311	0.0869
69	0.5455	0.0920
73	0.5585	0.1026
76	0.5708	0.1098
81	0.5832	0.1214
85	0.5956	0.1279
89	0.6016	0.1346
94	0.6095	0.1426

Table B.17: Data for Figure 6.3

	Total CO_2 data	
Day	Actual	Predicted
0	0.0000	0.0000
3	1.0235	0.5193
6	2.6010	1.1770
8	4.0067	1.7081
10	4.7290	2.3257
12	5.4416	3.0415
14	6.1121	3.8678
16	6.8247	4.8174
18	7.5696	5.9028
20	8.0911	7.1360
22	9.6459	8.5275
24	11.2783	10.0858
26	12.9724	11.8157
28	14.7344	13.7179
30	17.0017	15.7875
32	19.2367	18.0137
34	21.6109	20.3790
36	24.2960	22.8595
38	26.3982	25.4253
40	28.5457	28.0421
43	32.4066	31.9808
45	34.5282	34.5606
47	36.5105	37.0621
49	38.8134	39.4543
51	40.8799	41.7120
53	42.6906	43.8164
55	44.2745	45.7550
57	46.2146	47.5220
59	47.5686	49.1168
61	49.0294	50.5435
63	50.7720	51.8098
67	53.1106	53.9035
69	54.5487	54.7552
73	55.8475	56.1314
76	57.0816	56.9245
81	58.3222	57.8973
85	59.5595	58.4395
89	60.1555	58.8308
94	60.9523	59.1689

Table B.18: Data for Figure 6.4

Day	$^{14}CO_2$ data Actual	Predicted
0	0.0000	0.0000
3	0.0142	0.0913
6	0.2417	0.1963
8	0.3719	0.2747
10	0.4430	0.3607
12	0.5364	0.4549
14	0.6024	0.5578
16	0.7054	0.6702
18	0.7835	0.7929
20	0.8762	0.9266
22	1.0268	1.0721
24	1.1825	1.2302
26	1.3864	1.4017
28	1.5496	1.5874
30	1.7862	1.7881
32	2.0093	2.0046
34	2.2396	2.2376
36	2.4821	2.4877
38	2.6765	2.7556
40	2.8923	3.0417
43	3.3072	3.5056
45	3.5863	3.8383
47	3.9340	4.1897
49	4.3576	4.5594
51	4.7891	4.9470
53	5.1962	5.3516
55	5.6148	5.7724
57	6.1718	6.2080
59	6.6537	6.6570
61	7.1023	7.1176
63	7.6064	7.5878
67	8.6895	8.5489
69	9.1997	9.0350
73	10.2577	10.0066
76	10.9807	10.7250
81	12.1437	11.8805
85	12.7868	12.7498
89	13.4585	13.5571
94	14.2570	14.4673

Table B.19: Data for Figure A.1

	C/N Ratio 10:1		
Day	30% Moisture	50% Moisture	70% Moisture
0	0.0000	0.0000	0.0000
4	0.0012	0.0007	0.0013
6	0.0012	0.0010	0.0029
10	0.0016	0.0012	0.0110
11	0.0018	0.0023	0.0119
13	0.0018	0.0023	0.0153
14	0.0020	0.0027	0.0166
18	0.0020	0.0028	0.0216
21	0.0025	0.0031	0.0239
24	0.0028	0.0032	0.0296
25	0.0035	0.0033	0.0325
28	0.0045	0.0046	0.0370
31	0.0045	0.0046	0.0388
32	0.0045	0.0047	0.0397
35	0.0060	0.0063	0.0454
36	0.0060	0.0063	0.0479
39	0.0061	0.0063	0.0508
41	0.0064	0.0070	0.0558
42	0.0071	0.0075	0.0581
45	0.0071	0.0075	0.0595
47	0.0078	0.0076	0.0610
49	0.0085	0.0083	0.0627
52	0.0097	0.0088	0.0653
55	0.0114	0.0091	0.0676
59	0.0126	0.0095	0.0731
68	0.0172	0.0139	0.0819
71	0.0182	0.0169	0.0879
76	0.0189	0.0213	0.0975
80	0.0206	0.0237	0.1055
87	0.0226	0.0293	0.1202
90	0.0226	0.0300	0.1210
96	0.0272	0.0348	0.1323
105	0.0289	0.0392	0.1414
108	0.0304	0.0419	0.1429

Table B.20: Data for Figure A.2

	C/N Ratio 30:1		
Day	30% Moisture	50% Moisture	70% Moisture
0	0.0000	0.0000	0.0000
4	0.0012	0.0010	0.0153
6	0.0012	0.0012	0.0268
8			0.0304
10	0.0020	0.0023	0.0375
11	0.0020	0.0023	0.0399
13	0.0020	0.0024	0.0445
14	0.0025	0.0032	0.0470
18	0.0025	0.0069	0.0555
21	0.0028	0.0111	0.0605
24	0.0029	0.0157	0.0668
25	0.0031	0.0178	0.0690
28	0.0044	0.0210	0.0744
31	0.0044	0.0226	0.0770
32	0.0045	0.0247	0.0800
35	0.0058	0.0283	0.0855
36	0.0058	0.0288	0.0866
39	0.0058	0.0303	0.0906
41	0.0065	0.0319	0.0932
42	0.0072	0.0329	0.0949
45	0.0072	0.0338	0.0977
47	0.0084	0.0350	0.0999
49	0.0096	0.0363	0.1024
52	0.0161	0.0385	0.1062
55	0.0217	0.0401	0.1088
59	0.0257	0.0421	0.1132
68	0.0331	0.0491	0.1237
71	0.0348	0.0515	0.1265
76	0.0369	0.0540	0.1300
80	0.0399	0.0569	0.1333
87	0.0515	0.0623	0.1390
90	0.0548	0.0636	0.1404
96	0.0647	0.0704	0.1474
105	0.0737	0.0771	0.1532
108	0.0759	0.0795	0.1558

Table B.21: Data for Figure A.3

	C/N Ratio 50:1		
Day	30% Moisture	50% Moisture	70% Moisture
0	0.0000	0.0000	0.0000
4	0.0007	0.0006	0.0101
6	0.0010	0.0011	0.0169
8			0.0227
10	0.0013	0.0051	0.0302
11	0.0013	0.0060	0.0326
13	0.0013	0.0086	0.0367
14	0.0018	0.0113	0.0396
18	0.0018	0.0184	0.0498
21	0.0021	0.0211	0.0567
24	0.0021	0.0239	0.0654
25	0.0025	0.0249	0.0672
28	0.0038	0.0300	0.0734
31	0.0038	0.0328	0.0776
32	0.0041	0.0342	0.0798
35	0.0056	0.0387	0.0852
36	0.0058	0.0395	0.0859
39	0.0061	0.0427	0.0899
41	0.0061	0.0451	0.0930
42	0.0087	0.0464	0.0945
45	0.0108	0.0487	0.0975
47	0.0137	0.0505	0.1014
49	0.0166	0.0524	0.1043
52	0.0252	0.0562	0.1086
55	0.0296	0.0583	0.1112
59	0.0347	0.0616	0.1164
68	0.0539	0.0743	0.1269
71	0.0568	0.0775	0.1297
76	0.0648	0.0818	0.1327
80	0.0701	0.0844	0.1357
87	0.0807	0.0918	0.1410
90	0.0842	0.0941	0.1419
96	0.0946	0.1020	0.1482
105	0.1036	0.1079	0.1547
108	0.1053	0.1101	0.1571

Table B.22: Data for Figure A.4

	70 % Moisture				
Day	C/N 10:1	C/N 30:1	C/N 50:1	Day	No added N
0	0.0000	0.0000	0.0000	0	0.0000
4	0.0013	0.0153	0.0101	6	0.0182
6	0.0029	0.0268	0.0169	10	0.0284
8		0.0304	0.0227	14	0.0375
10	0.0110	0.0375	0.0302	18	0.0450
11	0.0119	0.0399	0.0326	24	0.0516
13	0.0153	0.0445	0.0367	28	0.0576
14	0.0166	0.0470	0.0396	33	0.0651
18	0.0216	0.0555	0.0498	38	0.0697
21	0.0239	0.0605	0.0567	42	0.0749
24	0.0296	0.0668	0.0654	49	0.0824
25	0.0325	0.0690	0.0672	54	0.0870
28	0.0370	0.0744	0.0734	60	0.0911
31	0.0388	0.0770	0.0776	65	0.0927
32	0.0397	0.0800	0.0798	74	0.0973
35	0.0454	0.0855	0.0852	79	0.0992
36	0.0479	0.0866	0.0859	88	0.1051
39	0.0508	0.0906	0.0899		
41	0.0558	0.0932	0.0930		
42	0.0581	0.0949	0.0945		
45	0.0595	0.0977	0.0975		
47	0.0610	0.0999	0.1014		
49	0.0627	0.1024	0.1043		
52	0.0653	0.1062	0.1086		
55	0.0676	0.1088	0.1112		
59	0.0731	0.1132	0.1164		
68	0.0819	0.1237	0.1269		
71	0.0879	0.1265	0.1297		
76	0.0975	0.1300	0.1327		
80	0.1055	0.1333	0.1357		
87	0.1202	0.1390	0.1410		
90	0.1210	0.1404	0.1419		
96	0.1323	0.1474	0.1482		
105	0.1414	0.1532	0.1547		
108	0.1429	0.1558	0.1571		