Studies Concerning the Fate of Atrazine and Chlorpyrifos in a Biobased Pesticide Waste Disposal System

by

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Studies Concerning the Fate of Atrazine and Chlorpyrifos in a Biobased Pesticide Waste Disposal System

Abstract

A disposal system for pesticide waste was examined for the ability to isolate waste pesticides onto lignocellulosic matrices, such as peat moss (PM) or steam-exploded wood fibers (SEW) by sorption processes, and to detoxify the pesticide during solid state fermentation (SSF) in contained bioreactors.

Emulsions formed from typical pesticide formulations acts as a barrier for pesticide sorption. Adding Ca(OH)₂ for demulsification of Dursban® 4E and filtering suspended particulates was the most effective steps for decreasing chlorpyrifos concentration. Activated carbon (AC) and rubber (R) were better at sorbing chlorpyrifos than the PM and SEW, even though the two sorbents were capable of removing approximately 95% chlorpyrifos from solution. Secondary columns of AC or R could be employed to further remove chlorpyrifos or similar pesticides from the waste water.

Atrazine and chlorpyrifos dissipation in field SSF bioreactors increased with higher bioreactor temperatures (65 °C). Dissipation time for 50% parent compound disappearance (DT₅₀) of atrazine in 1 m³, cylindrical (1 m diameter), and plastic bag (189L) bioreactors were 2, 14, and days, respectively. The chlorpyrifos DT₅₀ values for the same bioreactors were 59, 20, and 50 days, respectively. Chlorpyrifos did not volatilize outside the compost as expected, but was found condensed in the cooler outer regions of the bioreactor matrix.

Supplemental energy and fertilizer sources (corn meal, vegetable oil, and chicken manure) enhanced microbial activity and pesticide degradation in field bioreactors. The dissipation of atrazine was not enhanced by nutrient amendment in laboratory studies involving ¹⁴C-radiolabeled atrazine and chlorpyrifos at 40 °C over 16 weeks. The DT₅₀ values were 4 weeks for nutrient amended and unamended bioreactors. Nutrient amendment increased chlorpyrifos dissipation with DT₅₀ values of approximately 11 and 17 weeks for nutrient and unamended bioreactors but not in the atrazine bioreactors. Dissipation of chlorpyrifos was mainly through volatility and degradation, whereas atrazine dissipated by degradation and unextractably associated with the matrix.

It is important to keep pesticide waste bioreactors well aerated, moist, and mixed with additions of nitrogen and fresh biomass to facilitate the dissipation and detoxification of pesticides. The remaining residue can be land applied, land filled, or incinerated.
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CHAPTER 1 INTRODUCTION

Disposal of pesticide waste can be a dilemma for anyone who uses pesticides. Solutions to a pesticide waste problem may be as simple throwing a can of aerosol insecticide into the normal municipal refuse, but as complicated as determining what to do with thousands of liters of pesticide-laden rinsate that has been collected and stored from rinsing pesticide application equipment. If pesticide waste is mishandled or deliberately dumped, the potential exists that the pesticide will reach a water supply and adversely affect humans and non-target species. Pesticide contamination in groundwater and surface water occurs and concentrations can reach levels that constitute a health concern. It has been reported in the National Pesticide Survey Project Summary (EPA, 1990) that 4% of the rural wells in the U.S. are contaminated with at least one pesticide. However, less than 1% of the U.S. rural wells are contaminated with concentrations exceeding standards set for safe drinking. It has been suggested that most of the incidences of pesticide detection in groundwater are a result of non-point sources, including normal agricultural application practices (Hallberg, 1989). However, Graham (1991) reports that point source pesticide pollution, such as spills, may be as responsible for groundwater contamination as normal agricultural practices. The highest pesticide concentrations found in groundwater have been attributed to point source incidents of pesticide entry into the environment. Point source pollution of pesticides include improper application, deliberate spills, accidental major spills, back-siphoning, and making tank mixes and cleaning application equipment near well heads (Gustafson, 1993).

Large agricultural operations or chemical dealers can generate between 35,000 - 50,000 gallons of rinsewater a year, and it may cost them between $0.80 and $1.50 a gallon (1988 prices) to transport the rinsewater as a hazardous waste to a Class I landfill (Brosten, 1988). It is the Resource Conservation and Recovery Act (RCRA) that requires those who generate hazardous waste to store it and dispose of it in a proper and legal manner. The label on the pesticide container gives instructions for safe and proper application, storage, and disposal. By using a pesticide, the pesticide user is legally bound to instructions on the pesticide label. A common statement on pesticide labels reads, "it is a violation of federal law to use this product in a manner inconsistent with its labeling." Additionally, by law pesticide labels must have a statement concerning storage and disposal, and a common statement concerning disposal reads as follows:
"Do not contaminate water, food or feed by storage or disposal. Pesticides are toxic. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of federal law. If these wastes cannot be disposed of by use according to the label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance."

Therefore, pesticide users are completely responsible for the handling of their pesticide waste, because they will be held accountable for any improprieties concerning the misuse of the pesticide as governed by the pesticide label. Original pesticide containers are to be triple-rinsed into the application tank per instructions on the pesticide label. The containers can then be recycled or taken to the landfill. The pesticide label does not usually give suggestions on how to deal with pesticide-laden rinsate or wastewater. The lack of good information of rinsate disposal on the pesticide label has been a point of contention for those who have to deal with the waste - the pesticide users (Lounsbury, 1992).

Even though the pesticide label lacks suggestions for pesticide disposal, there are good methods and technology for dealing with the disposal of pesticide-laden rinsate. Of course, the best procedure to follow in dealing with pesticide waste is minimization of the waste generated. If all of the pesticide made for an application can be used, the only other pesticide waste to deal with is the rinsate from equipment washing. The rinsate can be collected, stored, and reused for making new batches of the same pesticide. If the resulting rinsate from equipment washing cannot be completely recycled, other means of disposal must be pursued.

There are acceptable ways to dispose of pesticide waste. A popular means of pesticide waste disposal involves circulating the collected rinsate through activated carbon filters (Brosten, 1988). Considerable research has gone into disposal systems that isolate pesticides from rinsate by sorption process, and several companies offer adsorption units employing activated carbon filters at reasonable cost to the pesticide user. One company, Westates Carbon (Los Angeles, CA), supply carbon filters at approximately $500; four of these filters were used to clean a pesticide lagoon in California. California with its legislated California Toxic Pit Cleanup Act required all lagoons to be closed and cleaned. The spent filters can be disposed of by Westates for approximately $350 each. Another acceptable methods of pesticide waste disposal includes incineration (Bridges and Dempsey, 1988).
Alternative or experimental pesticide waste disposal processes have been studied and include photolysis (Peterson et al., 1990), ozonation (Kearney, et al, 1990), hydrolysis (Kennedy et al., 1981), biological degradation (Hall et al., 1981), adsorption to biologically active carbon (Massey et al., 1992), and combinations of ozonation and biological degradation (Somich, 1990). Another disposal method involves bioreactors based on aerobic composting techniques (Snell Environmental Group, 1982; Hetzel et al., 1989; Mullins et al., 1989; Berry et al., 1992, 1993; California Agricultural Research Inc., 1992; Shanks et al., 1992). The highly biologically active state achieved during a composting situation can greatly accelerate the degradation of waste pesticides. The U.S. Army is using composting as an effective means to treat explosives contaminated soil (Williams and Myler, 1990). Explosives include 2,4,6 trinitrotoluene and several other related compounds. Explosives contaminated soil is incorporated into compost windrows and managed in similar manner as municipal solid waste (MSW) composting systems. The U.S. Army determined that the composting process was quite cost effective, costing approximately $100 to treat a ton of contaminated soil using the composting process compared to $250 to treat the contaminated soil by incineration.

A pesticide disposal system is being developed by an interdisciplinary team of researchers at Virginia Tech (Hetzel et al., 1989, 1993; Mullins et al., 1992Berry et al). The Virginia Tech pesticide disposal system involves two major steps which are illustrated in Figure 1. The primary step is the isolation of the pesticide phase in rinsate or wastewater through sorption processes and filtration. Lignocellulosic materials, such as peat moss, steam-exploded wood fibers, and steam-exploded peanut hulls are used in a batch sorption step with the pesticide-laden rinsate. The majority of pesticides are removed in the batch sorption step, but the aqueous phase is then passed through a column of similar lignocellulosic materials, and then through a series of tertiary filters of activated carbon. The water effluent, virtually devoid of pesticides, can be used in some non-potable agricultural practices, such as irrigation.

The remaining lignocellulosic materials with the sorbed pesticide phase is then taken to contained bioreactors and composted. The biologically active situation of composting has been successful in detoxifying pesticides through physicochemical reactions and biodegradation. In a peat moss based composting bioreactor, diazinon at an initial concentration of 32,000 μg/g dry matrix degraded to approximately 7 μg/g after 18 weeks at temperatures exceeding 50 °C. Preliminary research indicates that the Virginia
Proposed Use of Wastes

1. Spent Sorbent can be land applied, land-filled, incinerated, or disposed of in some other appropriate manner
2. Water can be used for irrigation or some other non-potable use

Figure 1  Virginia Tech Pesticide Waste Disposal System
Modified from Hetzel et al., 1989, Mullins et al., 1992, Berry et al., 1993
Tech pesticide waste disposal system is a promising technology with practical and useful applications.

The research for this dissertation was conducted with the sole concern to study how to further optimize the two stage Virginia Tech pesticide waste disposal process. The hypotheses of the dissertation research were that demulsification of emulsions would improve sorption of the pesticide phase, lignocellulosic materials will be good sorbents for pesticide sorption from solution, and that increasing bioactivity in the compost bioreactors will accelerate pesticide dissipation and eventual detoxification of the original pesticide waste.

Two main objectives supporting the hypotheses were the basis of this research. These objectives were focused on the two main stages involved with the pesticide waste disposal process: (1) isolation of pesticides dispersed in wastewater onto biobased lignocellulosic sorbents, (2) enhanced dissipation of these isolated pesticides in an aerobic and biologically active compost bioreactor.

The first objective was to study the effects of an emulsion on pesticide sorption behavior and to study the degree to which chlorpyrifos sorption occurs with a variety of biobased lignocellulosic sorbents compared to granular activated carbon.

The second objective was to study the fate of atrazine and chlorpyrifos in composting situations, and to improve the dissipation rates as affected by compost structure and management. Three specific areas of research were conducted to meet the two objectives as follows:

1. Demulsification and sorption experiments with chlorpyrifos formulated as an emulsion using Dursban® 4E and biobased sorbents, such as steam-exploded wood, peat moss, granular rubber, and granular activated carbon.

2. Field studies involving different compost structures and management to improve the biological activity of the compost. Additionally, studies were conducted to determine if these improvements to compost design and activity improved pesticide dissipation.

3. Small scale compost studies were conducted to correlate atrazine and chlorpyrifos dissipation with nutritive treatments. Respirometric methods were used to quantify oxygen consumption as an index of microbial activity.

4. Enclosed laboratory scale studies were conducted to determine the fate of radiolabeled $^{14}$C-atrazine and $^{14}$C-chlorpyrifos in compost bioreactors.
Atrazine and chlorpyrifos were chosen as the two pesticides to study in the research for this dissertation for several reasons. Atrazine and chlorpyrifos are commonly used pesticides in U.S. agriculture, therefore would present growers with a frequent need for disposal. Also, atrazine has been identified as the pesticide most commonly detected in groundwater samples (Hallberg, 1989; Furari, 1995). Chlorpyrifos is also found in groundwater samples, but because of its low water solubility (2 μg/g), its frequency of detection is much less than atrazine. Because of these facts, atrazine and chlorpyrifos are good representatives for research with the pesticide waste disposal system.
CHAPTER 2 REVIEW OF LITERATURE

2.1 Pesticide Use in the United States

Pesticide use in lbs/acre has decreased from a high of approximately 500 to 600 millions of pounds of pesticide applied annually between 1977 and 1983 to 485 million pounds of pesticide applied in 1987. This decline in overall pesticide use was probably due to better management practices and utilization of more effective pesticides requiring smaller amounts applied to give effective control (i.e. pyrethroid insecticides and sulfonyleurea herbicides) (Robbins, 1989). The pesticide market was estimated to be $26 billion in 1993, $11.4 billion for herbicides, $7.54 billion for insecticides, $5.46 million for fungicides, and $1.56 billion for nematicides and plant growth regulators (Klassen, 1995).

Herbicide use in U.S. agriculture is greater than other pesticide classes. The United States Department of Agriculture (USDA) estimated that approximately 170 million kg of herbicides and 45.4 million pounds of insecticides were applied in 1987 to crops grown in the United States (USDA, 1987). This represented approximately 65% of the total pesticide use being herbicide application followed by 20% insecticide and 15% fungicide application. The change from a predominance of insecticide use to predominant use of herbicides occurred in the mid to late 1960's. This has been attributed to the development of newer herbicides that significantly improved crop yield (USDA, 1987).

In 1986, approximately 90% of total herbicides applied in this country were applied to corn, soybeans, wheat, and cotton (USDA, 1986). In 1987 the largest percentage of total herbicide used was applied to corn (approximately 90.7 million kg) followed by herbicide applied to soybeans (45.4 million kg), and then wheat and cotton, both with approximately 9.1 million kg applied in 1987 (USDA, 1987).

In 1987, the largest insecticide application rates were applied to corn (25 million pounds) followed by cotton (6.8 million kg), soybeans (4.5 million kg) and wheat (0.9 million kg) (Robbins, 1989). Until 1982, the majority of insecticides were applied to cotton, but presently other crops receive more total insecticides. This change could be a result of better management practices and the switch to synthetic pyrethroid and sulfonyleurea compounds. Synthetic pyrethroids are more effective at lower concentrations than comparable amounts of organophosphates or carbamate insecticides. The same is
true for the sulfonylurea herbicides which are applied at grams per hectare versus kg per hectare of triazines.

There are many other agricultural crops and practices in the United States in which pesticides are used. These include alfalfa, nuts, potatoes, tobacco, tomatoes, tree fruits, and turfgrass. Pesticide use on these crops comprises only a small percentage compared to corn, cotton, soybeans and wheat. However, since many of these crops have special pest problems, they require a wide range of pesticide use patterns.

Pesticide use in urban pest management is also important. The pesticide market for this type of pest control is less than the agricultural use of pesticides, but the total urban pest control industry is estimated to be in the millions of dollars. Other areas where significant amounts of additional pesticides are used include human health and animal (livestock/pets) health, greenhouse plants, ornamentals, right-of-way, and aqueous pest control.

The cumulative use of pesticides is diverse and can be related to just about any pest inhibiting or affecting human activity. The pesticide use patterns change over time because of newly developed pesticides, legislation, opening markets and other factors. The trend is to minimize pesticide use by applying more effective pesticides and following better management practices (BMP's). It is not likely that pesticide use will be eliminated in the near future, but environmentally sound and responsible use of pesticides is and will continue to be the goal of the pesticide user.

2.2 Pesticide Use in Virginia

Overall pesticide use in Virginia has followed national trends. Virginia has a variety of crop types and the pesticide use patterns reflect growers' needs. In 1993, the major cash crop in Virginia was tobacco ($180.8 million in receipts) followed by soybeans ($74.1 million), peanuts ($52.3 million), wheat ($37.9 million), hay ($37.9 million), tomatoes ($34 million), grain corn ($32 million), apples ($30.5 million), and others (Va Ag Stat Service, 1993). On the other hand, the top acreage crop in Virginia in 1993 was hay (490,000 million hectares), including alfalfa, followed by soybeans (190,000 hectares), corn (115,337 hectares), wheat (103,000 hectares), and peanuts (38,000 hectares) (Va Ag Stat Service, 1993). Virginia has a variety of other important crops which have pest problems requiring pesticide application.
There are a variety of pesticides available for grower use. However, determining what the predominant herbicides or insecticides are used and in what crop is difficult. The primary reason is that no centralized pesticide/crop reporting system is available for Virginia. Estimations of Virginia pesticide use can be obtained through information from extension agents, the scientific community, pesticide dealers and extrapolation from surveys by other regions or States. The following Virginia pesticide use patterns were constructed from those sources and is presented as qualitative summary. The major herbicide use in Virginia involves alachlor, atrazine, butylate, dicamba, S-ethyldipropylthiocarbamate (EPTC), glyphosate, linuron, metolachlor, paraquat, simazine and 2,4-dichlorophenoxyacetic acid (2,4-D); predominant insecticide use in Virginia include acephate, aldicarb, azinphos-methyl, carbaryl, carbofuran, chlorpyrifos, diazinon, malathion, methomyl, and permethrin; predominant fungicides used in Virginia include captan, mancozeb, metalaxyl, sulfur, and tetramethylthiuramdisulfide (thiram).

2.3 Pesticides in Groundwater

There has been a growing public and scientific concern over groundwater contamination with hazardous chemicals, including pesticides. Since some pesticides are toxic to mammals, there are probable health risks when pesticide-laden water is consumed by humans.

The routes of entry by pesticides into the groundwater are from either point or non-point sources. Point source pollution may occur where specific activities result in an accidental or deliberate release of pesticides onto the ground which make their way into the groundwater. Some important point sources include some manufacturing sites, storage areas, transfer areas, formulation centers, transport, and mixing and disposal sites. In intensive agricultural areas, such as the corn belt, approximately 1500 different point sources have been identified (Hallberg et al., 1986; Long, 1988; Hallberg, 1989). On the farm, major point sources consist of spills, back-siphoning, and deliberate disposal. The result of point source contamination can be considerable and the amounts of pesticide released into the environment may be high, but the contamination is usually localized. Non-point pollution originates from chemical input over a large area rather than from a single source as in the case with point source pollution. Non-point sources, such as
normal farming practices, are widespread and probably constitute the majority of overall groundwater contamination by pesticides (Hallberg, 1989).

The accepted hypothesis on how pesticides enter groundwater from non-point sources through the soil profile is based on preferential flow theory. Micropores and macropores exist in the soil structure. Water can move through the macropores at rates higher than through compact soil and micropore regions. Macropores can be envisioned as the cracks in clay, for example, but may extend to many soil types and states of hydration. There are several pesticides that do not easily move into the groundwater because they bind to charged sites on soil particles (e.g. clay) due to their ionic characteristics (e.g. paraquat). The non-ionic dinitroaniline herbicide trifluralin is relatively immobile in micropores, but is found moving in macropores (Hallberg, 1989). Atrazine, metolachlor and dieldrin have been found in macropores of the soil profile to depths of 1.5 meters (Hallberg et al., 1986). However, they were not found below 0.5 M in the soil samples devoid of macropores.

It appears that many pesticides are soluble enough to present a hazard in groundwater even at low concentrations (parts per billion; ppb or μg/L). At one time it was believed that relatively insoluble pesticides would not be found in groundwater. However, atrazine with a water solubility of approximately 33 μg/g has been the most frequently found pesticide in wells tested (Hallberg, 1989). Evidence now suggests that only pesticides having a water solubility below 1 μg/g do not pose a problem for groundwater contamination (Gustafson, 1993). Pesticides having a water solubility above 1 μg/g can reach the groundwater supply by leaching through the soil or direct entry into the groundwater supply. Some pesticides do not become a groundwater problem because they are easily degraded by chemical means (e.g., hydrolysis) under the normal range of soil conditions or are rapidly biodegraded by soil microorganisms (Kearney and Karns, 1987). Alachlor is fairly water soluble (240 μg/g), is non-ionic, and is used in most situations at twice the rate of atrazine, and during the 1980's more pounds of alachlor were applied in the U.S. than any other pesticide (Gustafson, 1993). So, it would seem probable that alachlor should have a high potential for groundwater contamination, but, even though alachlor is detected in wells, the concentrations are generally lower than the acceptable contaminant level of 2 ppb. Biodegradation and biotransformation of alachlor (both aerobic and anaerobic) appears to be the reason alachlor does not usually present a groundwater contamination problem (Beetsman and Deming, 1974). Information is available on pesticide soil half-lives which indicate the level of chemical and/or
biodegradation in various soil types. These half-life values are usually variable between different soils and chemical conditions, but the general trends observed can be used for prediction purposes.

Trends of the herbicides used in Virginia agriculture which present a problem with groundwater resemble national trends. Atrazine is the most commonly detected pesticide in groundwater samples throughout the United States followed by alachlor (Gianessi et al., 1986; Hallberg, 1989; Funari et al., 1995). Butylate appears to be third in groundwater detection followed by 2,4-D/2,4-DB, metolachlor and trifluralin. Other herbicides detected at quantities above those set for health standards are dicamba, metribuzin and prometon (USDA, 1987; Louis and Vowinkel, 1989).

Nationally, carbofuran is the most common insecticide detected in groundwater, but chlorpyrifos, carbaryl, fonofos, phorate, aldicarb and dimethoate have also been detected (Hallberg, 1989). Other insecticides, such as disulfoton, ethylendibromide (EDB), fenamiphos, methomyl and oxamyl have been detected in groundwater at various places in the United States and these could pose a problem in Virginia since they are used in Virginia agriculture (Anderson et al., 1986; Halberg et al., 1986; Kessler, 1987; USDA, 1987).

If pesticides are used correctly then there should be minimal problems in groundwater and residues on food. The Safe Drinking Water Act under the EPA's regulation, requires that maximum contaminant levels (MCLs) be set for pesticides in the drinking water supply. Many of these MCL values are determined by considering the physicochemical nature of the pesticide in the environment and its acute and chronic toxicity to humans and non-target species. The MCL values are similar to the acceptable daily intake (ADI) allowable in food for human consumption. The ADI values are related to no observable adverse effect limits (NOAEL) determined in test animals, but are less due to a safety factor. The NOAEL limit is more clearly defined as the limit where there is no significant difference between the test animals treated with certain amounts of a test compound and those test animals not treated. Effects may include tissue abnormalities as well as the test animals' behavior.

The limits of pesticides allowable in food originate from the Delaney clause, which a legislative addition to the Federal Insecticide, Fungicide and Rodenticide Act of 1947 (FIFRA). However, in the recent years, there has been congressional debate concerned with the Delaney clause and food quality. The original clause stated that any additive that has been found to cause cancer in laboratory animals and found at any concentration will
not be tolerated in food or drinking water. Partially because instrumental detection levels become more sensitive over time, the EPA began using a risk standard for the acceptable degree of cancer in laboratory animals. The court system prohibited (1995) the EPA's use of the risk standard, reverting to the original interpretation of the Delaney Clause. The EPA's interpretation of risk stated that pesticide concentrations in drinking water and in processed food be below the levels needed to cause 1 in 10^6 cancer cases in laboratory animals. Others believe that any pesticide that is consumed at any level is wrong. However, Dr. Bruce Ames, a University of California toxicologist, the originator of the " Ames test" for determining mutagenicity of chemicals, stresses that humans are subjected to a variety of natural carcinogens with more mutagenetic capabilities and found in everyday food at greater levels than pesticides (Ames, 1991). The Food Quality Protection Act of 1996 was passed on August 3, 1996, which removed the Delaney Clause and replaced it with the requirement to set tolerances of pesticides in food and raw commodities. The tolerance levels (maximum pesticide residue allowed on a commodity) now calls for "reasonable certainty of no harm from aggregate exposure". It is expected that a safety factor (100 fold proposed ) will be placed on no observable effect levels (NOEL) from animal toxicology testing. Of special concern are setting tolerances in infants and small children. Effects of detectable amounts of certain food additives may be more pronounced in children due to their smaller size.

2.4 Alternative vs. Conventional Agriculture

Because there is concern with pesticides in groundwater and residues on food, there is a strong desire by consumers, growers, regulatory groups, and politically-oriented groups to reduce or eliminate the use of pesticides. The pesticide dilemma is a complex problem with a range of opinions including vehement proponents and opponents of pesticide use. The proponents and opponents of pesticide use do generally agree that using smaller amounts of more effective pesticides is desirable for reducing the environmental impact.

Since growers are businessmen, their strategy is oriented towards minimization of input costs and maximization of yields and profits. New farming management practices and pesticide usage allow the achievement of low chemical input while maintaining higher yields. Some of these practices include rotating crops to prevent insect problems in a
monoculture and supply natural fertilizer to the ground. Pest population monitoring is used to assist in deciding when to spray, which minimizes applications and could eliminate preventative spraying for a problem that may not exist. Recent regulatory laws and new pesticides have been designed to constitute a more environmentally safe use of pesticides and minimize pesticide application due to their increased efficiency. As a result, the grower can apply less quantity to the crops, observe better results and generally deal with a safer compound, both environmentally and to human health.

There is an increasing market for the "organically" grown crops. This means food that has been grown without the use of pesticides or only with "natural pesticides". Natural pesticides are compounds that are usually obtained from a plant source. Rotenone, derived from the roots of derris plants is a good example of a "natural insecticide", but rotenone is also considerably toxic to vertebrates (rotenone LD₅₀ of 60 µg/g versus 8 µg/g for carbofuran). Because there is an increased demand for these "organically" grown foods, there is an increasing need for pesticide-free or low-input agriculture. These organic foods usually are more expensive to the consumer because of the decreased yield and increased labor and pesticide costs, which reduce the profit margin for the grower. At this point in time "organically" grown food is a practice that is reducing synthetic chemical input, but it remains a specialty market. With reduced pesticide use, there usually is an increase in pest control labor (Robbins, 1989). In any case, there is an awareness that has encouraged alternative practices for agriculture that minimize or eliminate pesticide use.

In conclusion, there is a movement away from the liberal use to a more judicious pesticide use, because of many factors, including economic, efficacy, resistance, and environmental awareness. Growers are using more effective and smaller quantities of pesticides. They are adapting low input and integrated pest management practices because of public health, environmental safety concerns and economic factors.

2.5 Pesticide Waste and Disposal Options

Pesticides are meant to disrupt an organism's physiology in a way that immediately or eventually kills it. Because humans and non-target organisms possess many of the same biological processes that are affected in the target pest, pesticides can be quite poisonous to humans or non-target beneficial species. Therefore, it is important to manufacture,
store, formulate, transfer, apply, and dispose of pesticides properly without spills or excess amounts which might reach non-target sites, such as groundwater.

Invariably, pesticide spills will occur at all levels of pesticide handling, which can be of major proportions. Besides spills, the largest source of pesticide waste comes from excess pesticides after application and from storage of outdated or unusable pesticides. The Resource Conservation and Recovery Act (RCRA) of 1976 dictates that pesticide users take necessary precautions to minimize the amount of pesticide left after application. Any generator of hazardous waste is required to inform the EPA and be compliant with facility standards. However, the act did not recommend specifics of compliance.

The most important way to dispose of pesticide waste is to reduce the amount of pesticide waste before it occurs. The widely distributed Farm Chemicals Handbook (1995) recommends the following guidelines adapted from Ozkan and Wilson (1989) to reduce pesticide waste: [1] purchase only what will be used, [2] improve application accuracy, [3] eliminate leftover spray mixture, [4] rinse containers immediately, [5] reduce rinsate, [6] modify spray equipment, and [7] choose pesticides packaged in returnable/refillable containers. If too much concentrated pesticide is purchased, then leftover amounts may remain in storage for years, and, in the future, there may be a need to dispose of it as pesticide waste. It is important to ensure that the application equipment is applying the pesticide amounts correctly. If the amount of pesticide applied is greater than desired, it may pose a threat as run-off to nearby surface water or reach the groundwater supply. It is possible to eliminate leftover pesticide amounts from an application by closer scrutiny to the calculations used for mixing the needed volume of formulated pesticide to apply. Spent pesticide containers should be triple-rinsed into whatever pesticide application apparatus is being used. Specially designed nozzles have been designed to aid in the efficient removal of pesticides from the container. Many states, counties, and pesticide distributors do have container disposal programs (Allison, 1992). Once again, the most effective means to control pesticide waste is to reduce the amount of waste that is generated. To assist in minimizing pesticide waste, some pesticide companies and dealers have bulk pesticide containers and the pesticides are put directly into the application tanks at the prescribed amount. The pesticide company or dealer becomes responsible for container disposal rather than the grower. Also, a nozzle injection system of pesticide application is available for some types of applications which eliminates practically all waste by simultaneously mixing and spraying. Even if care is taken to utilize all the pesticide, there will still be waste rinsates from flushing or cleaning
the application equipment or original pesticide containers. Many pesticide companies now provide formulation packaging that can be thrown directly in a tank mix where it dissolves. This eliminates the container problem and ensures that the proper portion is administered for the correct pesticide concentration.

The United States Environmental Protection Agency (EPA) recommends the following management practices for dealing with empty containers in the order of preference: container minimization, container reconditioning and recycling, and container disposal after proper rinsing, collection, and proper disposal of any pesticide waste (Fitz, 1992). Excess pesticide can be discharged on boundary vegetation at the prescribed label rate. However, it is illegal to deliberately dump any excess pesticide on the ground. The amount of intentional pesticide spillage is not known and may well constitute a large portion of point-source pesticide released into groundwater (Hallberg, 1989).

Containers are one source of pesticide waste, but pesticide-laden wastewater from rinsing tanks and equipment constitutes a disposal problem. Brower (1986) estimated that tank rinsates can contain 10% of the original pesticide concentration. Pesticide concentrations in sprayer tanks can range between 2,000 and 16,000 μg/g (Woodrow et al., 1989). Some pesticide users generate 50,000 gallons of pesticide-laden wastewater in a season (Brosten, 1988). It is required by law that the pesticide-laden wastewater be stored until proper disposal, but it is the generator of the pesticide waste that has to decide how to dispose of it. The most pragmatic way to deal with pesticide-laden rinsewater is to recycle it back for use in making the next tank mixes of the same pesticide.

Probably the most common method to isolate the pesticide phase from contaminated water involves sorption to activated carbon. The spent activated carbon with the sorbed pesticide is stored, land-filled, soil-incorporated, or incinerated. Incineration of isolated pesticides is a popular means for the final step in the disposal of pesticide waste (Dillon, 1981; Bridges and Dempsey, 1988). The cost of incineration has been minimized through cooperative means, as in the example of eight Ohio pest control operators (PCO's). They collected 40 fifty gallon barrels of pesticide waste and paid an environmental firm to collect and incinerate the waste (Pest Control, 1990). Included with incineration methodology is an increasingly available disposal technology involving high temperature plasma energy to handle hazardous waste, such as medical waste and low-level radioactive waste (Vanguard Research, Inc., Fairfax, VA). The product of the 2,000 to 3,000 °F process is a benign glass that does not leach toxicants and is a considerable reduction of the original waste volume that is to be landfilled.
Activated carbon is one of the best and cost-efficient sorbents used for clarifying water from household use to industrial levels. The most important factor that makes activated carbon such a good and widely used sorbent is its large surface area, having between 500 and 1000 m² per gram, which translates to about 100 acres per pound of activated carbon (Lehr, 1991). Isolation of hazardous compounds is the primary role of activated carbon, but incineration or burial in a Class I hazardous landfill is required for any contaminated activated carbon (Brosten, 1988).

Several techniques for sorbing pesticides to activated carbon have been developed and employed. Kobybinski et al. (1984) developed a recirculating system through activated carbon. They showed good removal of several pesticides with varying water solubilities. They reported a cost to assemble the device at approximately $3000 in 1981. Massey et al. (1992) demonstrated a biologically active system involving activated carbon that was effective in degrading 2,4-D from contaminated water. The Wilbur-Ellis Company offers a service to reduce the volume of rinsewater by passing the rinsewater through activated carbon filters. Pesticide-laden rinsewater in volumes of approximately 50,000 gallons can be reduced to three 55 gallon drums of contaminated activated carbon that can be incinerated or taken to a Class I landfill (Brosten, 1988). The Wilbur-Ellis Company in 1988 estimated that the cost to a grower or dealer would be between $30,000 and 40,000 to install a concrete collection pad, plumbing, and filtration equipment for the disposal process. The cost is reduced to about $18,000 if a concrete collection pad is already in place. However, the majority of growers have not constructed elaborate wash pads due to the expense and their level of pesticide use (VAC & SFA, 1990). It appears that only growers who have large agriculture operations can justify the expense of wash pads and they usually find that it is an effective and economical means for controlling pesticide waste.

Collection and disposal of pesticide waste exists in many forms, but the pesticide user still has to pay a considerable amount for the transportation and service (Dillon, 1981). Several pesticide manufacturers, dealers and large-scale applicators have installed pesticide collection pads that are usually made of concrete. These are designed to collect pesticide wastes and rinsates resulting from daily equipment clean-up operations. The pesticide wastes are transferred into a collection reservoir and disposed of or reused in a proper and legal manner. Large operations or dealers generate between 35,000 to 50,000 gallons of rinsewater a year and it may cost between $0.80 and $1.50 a gallon to transport as a Class I hazardous waste to a Class I landfill (Brosten, 1988).
In addition to the disposal methodology involving adsorption of the pesticide phase to activated carbon and subsequent incineration or landfilling, there are other adsorption methods involving a secondary biodegradation stage. The primary sorption phase can be to activated carbon or some other sorptive material, such as peat moss. Atkins (1972) used a biofilm apparatus that absorbs the pesticides as they flow over the biofilm and then are biodegraded. Dalmacija et al. (1992) described a bioactive system for degrading pesticides sorbed to activated carbon. Maintained on the activated carbon is a biofilm that is produced by attached microorganisms. Gamma benzene hexachloride or lindane (a chlorinated insecticide) and quinlaphos (an organophosphorus insecticide) in contaminated water were shown to degrade due to the biofilm present. Nye (1984) developed a disposal system by which the pesticide phase from wastewater goes through a flocculation/sedimentation stage and then the supernatant is pumped through activated carbon filters. The isolated pesticide phase on the activated carbon and contained in the sediment has to be disposed of as hazardous waste. A similar disposal system developed by Hetzel et al. (1989) and Mullins et al. (1992) involves isolating the pesticide phase from a primary batch sorption process and secondary column sorption process using lignocellulosic materials (i.e. peat moss) as the sorbent. The pesticide phase isolated on the sorbent can then be placed into bioreactors in a composting situation whereby degradation occurs under highly biologically active conditions. Mullins et al. (1989) demonstrated that concentrations between 4000 and 32,000 μg/g diazinon placed into a composting environment for 18 weeks was dramatically reduced to concentrations approximately 1 and 7 μg/g, respectively. Their pesticide waste disposal system is designed to remain a cost-effective means to handle pesticide waste for the pesticide user who does not have a large operation. Other methods that are available for pesticide waste disposal have been developed by industry, but few practical methods exist at an affordable cost and use for the small-scale pesticide user to utilize. Effective treatment options may not be used due to problems with proximity to the pesticide applicator.

Other biologically active systems involving composting or activated sludge is another area of research of degrading pesticide waste. Racke and Frink (1989) demonstrated degradation of carbaryl by sewage sludge composting. Nye (1980) has developed a pesticide waste disposal system where the pesticides in solution are flocculated in a sewage treatment plant and then the sediment present in activated sludge is biodegraded and can be discarded. Shibamoto and Seiber (1992) have developed in co-operation with Advanced Manufacturing and Development (Willits, CA) have studied
the fate of waste pesticide from rinsewater in the company's Bio-Gest biological digester. Approximately 75 L of trifluralin, atrazine, carbofuran, and diazinon at 1000 μg/g at different times were allowed to circulate from a sump pump over an enclosed bed of horse manure. The leachate was then recirculated back over the horse manure for 16 days. Temperatures reached 45 °C and the half-lives of the trifluralin, atrazine, carbofuran, and diazinon were 34, 69, 20, and 34 hours, respectively. They intend to make a larger scale system capable of handling hundreds of liters a day of pesticide-laden wastewater. It is interesting that they did not comment on how they disposed of the spent horse manure matrix. They did indicate that horse manure could be added continuously to the Bio-Gest reactor.

During the early 1980’s, several researchers developed large concrete pits to hold pesticide waste and allow for volatilization, as well as abiotic and biotic degradation of the pesticides. Hall (1984, 1988) at Iowa State constructed concrete pits (3.66 by 9.14 by 1.22 meters) with removable covers, which contained water, gravel, and soil. Junk and Richard (1984), also at Iowa State, showed that over eight years of using the covered evaporation pits designed and constructed by Hall et al. (1981) were effective for disposal of 30,000 to 90,000 L of pesticide-laden wastewater containing a plethora of pesticides (24 to 40 different pesticides). Because the pits were open for volatilization of the pesticide phase, they recorded the concentration of pesticide residue in the surrounding air, but concluded that it did not pose a threat to human health at the recorded levels. Claunch (1988) described a method called the toxic substance solvent evaporator (TSSE), which involves a wicking action to accelerate evaporation of the aqueous portion, any carrier solvents, and the toxic substances present. The result is a reduction in the volume of the bulk waste, which can be handled more easily, and further methodologies can be used to degrade the toxic portion. Another method involving evaporation pits from holding pits is reported (Winterlin et al., 1984). They described a disposal method by which various pesticides from equipment and container rinsewater could put into covered 6.1 by 12.19 by 0.91 meter concrete pits containing soil and water with amendments of lime Ca(OH)₂. Through the processes of photodegradation, abiotic and biotic degradation, and vaporization, the pesticide levels decreased and the air around the pit was not considered to be toxic.

Similar methods involving pits or containers employing abiotic and biotic degradation have been developed over the years. Junk et al. (1984) reported that several pesticides (atrazine, alachlor, 2,4-D, trifluralin, carbaryl, and parathion) were degraded to
various extents in a biologically active system. Their disposal system involved containers of approximately 60 L of water 15 kg of soil. Degradation was quite rapid for 2,4-D, but did not occur for atrazine. Treatments of aeration, nutrients, flocculants, and buffering agents had questionable effects on the degradation of the pesticides.

The EPA has examined the potential use of enzymatic columns having pesticide affinities as means for pesticide degradation. The enzymes were isolated and purified from microorganisms (Munnecke, 1976, 1980; Landis et al., 1989).

Another promising pesticide waste disposal process involves genetically engineered microorganisms (Bridges and Dempsey, 1988). These new "superbugs" may accelerate the degradation process in bioreactors or soil because they would have an increased affinity and metabolic potential for predesignated compounds. The genes that code for the production of the specific degradative enzymes are bioengineered by cloning techniques and the result is microorganisms that produce larger than normal amounts of specific enzymes for compounds found in chemical waste.

Examples of some of these other methods of pesticide waste disposal are based on chemical/physical methods, which include: a hydrolysis apparatus developed to degrade compounds to safe byproducts (Kennedy et al., 1969; Dennis, 1972); a photolysis method that uses high levels of UV light to degrade pesticides (Mitchell, 1961; Crosby, 1977; Plimmer, 1977); and ozonation methods that utilize UV light and ozone gas to decompose the pesticides to CO₂ and H₂O (Bridges and Dempsey, 1988).

2.6 Isolation of Pesticide Waste

2.6.1 Formulations

Technical-grade pesticides can be formulated in many ways which include liquids, emulsions, solids, microencapsulation and edible baits (Ware, 1994). Different formulations may enhance the ability for the pesticide to be applied efficiently, reach the target organisms effectively and be absorbed and translocated within the target pest organism at a desired rate. If the pesticide is water soluble, it is usually packaged in a concentrated form (solid or liquid) and it is diluted with water prior to application. If the pesticide is not water soluble, it may be formulated as an emulsifiable concentrate (EC), a prepackaged emulsion, a wettable powder (WP), microencapsulated, granular (G) or others. These formulated products may also contain adjuvants that promote spreading,
sticking, and toxicity to target pest organisms. Different formulations are chosen usually because it is important to consider where the pesticide needs to best control the target pests. Also, economics, safety, mode of action, and, in general, enhancing the efficacy of the pesticide are important in the consideration of formulation development.

Each crop or human activity has its own array of associated pest species. Some pests are present in other crops or geographical regions, but the specificity and complexity of the pest population may differ between localities. A variety of pesticides with different formulations exist to control these pests. In general, if the pest is to be treated on the foliage then a liquid spray is used (soluble pesticide, emulsion, wettable powder and others) to ensure that the pesticide is evenly applied over a surface. This controls pests on a contact basis, but also, as with the case with many herbicides, provides foliar uptake.

If the pest is to be treated in the soil, a granular, liquid microencapsulation or gaseous form is incorporated into the soil. The pesticide present in the soil then comes in contact with, or is translocated to the pest species. Soil fumigants are used to effectively infiltrate the soil and control soil-borne pests. Certain herbicides have been developed to be absorbed through the root system. Also, many insect pests overwinter in the soil or perform their destruction at the soil level and need to be treated in the soil. Examples of soil-incorporated insecticides are carbofuran and chlorpyrifos, and both may be formulated as granular particles.

In conclusion, pesticides are formulated in various ways to control pest organisms by different routes, such as by direct contact or exposure in the soil. For example, the herbicide atrazine is formulated in various ways to be either foliarly-applied or soil-incorporated and which formulation is chosen depends on where the pest control is desired.

2.6.2 Emulsions

Emulsions are the predominant pesticide formulation (Becher, D., 1985; Ware, 1994). Most pesticides are fairly hydrophobic and to be delivered or applied, they must be solubilized because water is the primary extender used in application. Surfactants which are added to insoluble pesticides allow for emulsion formation with the addition of water. The pesticides contained in soluble micelles create a fairly stable and homogeneous solution. Emulsifiable concentrates are designed so that they form emulsions quickly, are quite stable in water, do not contain adjuvants which are toxic to non-target organisms,
and are reasonably biodegradable. Emulsions are defined as a heterogeneous system, consisting of at least one immiscible liquid dispersed in another in the form of droplets (Becher, P., 1985; Shinoda and Friberg, 1986). The droplet diameter, in general exceeds 0.1 μM. The IUPAC further defines an emulsion into two forms (Shinoda and Friberg, 1986): one being oil in water (O/W) where droplets of an organic liquid or the hydrophobic "oil" are suspended in an aqueous medium; and the reverse of water in oil (W/O) where water droplets are suspended in an organic "oil" phase. Milk is a typical O/W emulsion. Petrochemical companies consistently have problems with extracting oil from the earth in the form of W/O emulsions. Pesticide emulsifiable concentrates generally form O/W type emulsions, but pesticide sprays applied from the air usually are W/O emulsions to prevent evaporation of the drops before they reach the ground (Becher, D., 1985).

2.6.2.1 Emulsion Types

Emulsion types may be distinguished on the basis of relative stability and droplet size. Microemulsions differ from macroemulsions by being more stable and having smaller droplet sizes. Microemulsions are transparent to translucent and have particle (micelle) sizes below 1400 Å (Prince, 1977). They are thermodynamically stable and will not separate or settle over time or with normal temperature fluctuations. Conversely, macroemulsions are emulsions that have micelle sizes above 0.1 μm and appear "milky" white. These macroemulsions are not thermodynamically stable, but are kinetically stable meaning the solution needs to be agitated to stay an emulsion. They will separate over time if allowed to settle and the rate of settling is increased with decreasing temperatures (Becher, D., 1985). Most agricultural emulsifiable concentrates form macroemulsions, but there are a few which form microemulsions. The first pesticide microemulsion was a chlordane formulation (Prince, 1977). Another microemulsion that is presently used is a mixture of butylate and atrazine (Anon., 1982). Becher, D. (1985) suggested that the reason more formulations are not developed as microemulsions has to do with economics. Evidently, the cost of adjuvants and the research leading to microemulsions is high and that cost prevents them from being competitive with contemporary macroemulsions.
2.6.2.2 Emulsion Components

Agrichemical emulsions differ from the normal O/W emulsions, such as milk, or W/O emulsions in that they have surfactants associated with the emulsion structure that enhance the micelle formation and stability. The composition and structure of emulsifiable concentrates are complex as well as variable. There are usually three to five surfactants blended together in specific quantities. It has been reported that as many as 12 different surfactants are incorporated in one pesticide formulation (Lindner, 1974). The minimum emulsion formulation contains two phases, one being lipophilic and the other hydrophilic. The pesticide phase is usually contained in the internal hydrophobic phase and is surrounded by a surfactant superstructure with an external water phase. The micelle superstructure can be one or many layers of surfactant matrices.

Various surfactant types can be used to solubilize the pesticide phase. Cationic surfactants are one type having a hydrophilic cationic region and a hydrophobic tail. Cationic surfactants are not used in agricultural emulsions to any great extent because they are expensive and have a considerable amount of biological activity. Hydrophilic surfactants are usually anionic in nature rather than cationic (Lidner, 1974). These usually are the oil-soluble types where the most frequent one used appears to be calcium dodecylbenzene sulfonate. The non-polar portion of the anionic surfactant incorporates the hydrophobic pesticide possibly contained in a nonionic surfactant internal to the micelle. The hydrophilic portion of the surfactant interacts with outer surface of the micelle and the water phase.

Many pesticides are hydrophobic enough to require a nonpolar solvent, such as aromatic hydrocarbons (Becher, D., 1985). Nonionic surfactants are used when the pesticide is solublized in a nonpolar solvent. The available nonionic surfactants are numerous and most are not biologically active.

2.6.2.3 Emulsion Formulations

Emulsions are formed in different ways, but a general scheme is that two immiscible liquids are mixed together. The interface between the two phases becomes distorted and droplets form. Which phase forms droplets, and size and numbers of droplets, depends on the quantity of each phase and their respective physical
repulsion/attraction characteristics. The surfactant reduces the surface tension of the formed micelle. Emulsion stability depends on the surface tension, size of the micelle and the respective physical chemistry of each phase. The hydrophilic/lipophilic balance (HLB) scale was developed by ICI Americas to compare emulsion properties (Griffin, 1949). It is an empirical test that involves pouring the surfactant mixture into a tube containing a solvent. The HLB of the surfactant mixture is determined by emulsion formation, morphology and stability which depends on the solvent nature. Another indication of the nature of emulsions and stability involves the phase-inversion temperature (PIT). Because the emulsion rheology is dependent on temperature, the HLB varies for a given surfactant system with temperature. The temperature at which a surfactant system changes from forming an O/W emulsion to forming a W/O emulsion is the PIT. In other words, the PIT is the temperature where the lipophilic and hydrophilic nature of the surfactant system just balances. The PIT is more easily determined than the HLB which appears to have variable results (Ross and Morrison, 1988).

Another emulsion parameter is the critical micelle concentration (CMC). The CMC is the minimum concentration of the surfactants in the surfactant mixture that is required for micelle formation (Ross and Morrison, 1988). The surfactant concentration is higher than the CMC in agrichemical emulsion because stability has to be insured in all types of water (Becher, D., 1985).

2.6.2.4 Emulsion Stability and Demulsification

Emulsion stability is based on many factors and the DLVO theory states in general terms that the emulsion stability is determined by the sum of attractive and repulsive forces between individual particles of the emulsion superstructure (Ross and Morrison, 1988). DLVO is an acronym for the names of the four scientists that developed the theory as follows: B.V. Derjaguin, L. Landau, E.J.W. Verwey, and J.Th.G. Overbeck. The forces involved with emulsion stability are electromagnetic and gravitational in origin. Several of these are electrostatic (coulombic and induction forces), electrodynamic (dispersion forces), electron or proton donor-acceptor interactions), repulsive overlapping of electron clouds, mass of the micelle and gravity (Ross and Morrison, 1988).

An interesting production technique has come about because of emulsion technology. Microencapsulated pesticides are pesticides contained within a polymer shell.
They are suspensions, but the encapsulation is formed in an emulsion type reaction. The internal pesticide is one phase and the surrounding coat is a polymerized micelle (Vadegaer, 1974). A decrease in contact toxicity for handlers are one of the advantages of microencapsulated pesticides. Another advantage by using microencapsulated pesticides is that the coating acts as a "timed release", which increases the residual effect and requires fewer applications.

Demulsification is the disruption of the surfactant-micelle integrity or superstructure. The disruption can be achieved in various ways that are either mechanical, thermal, chemical or a combination (Ross and Morrison, 1988). Biological degradation is another way to disrupt an emulsion (Lissant, 1983). Demulsification can occur in several ways, but settling by sedimentation, disruption of the micelle superstructure or a combination are the predominant ways. Demulsification by sedimentation occurs in 2 basic steps; coalescence and sedimentation (Becher, D., 1985). Coalescence involves micelle aggregate formation. This process depends on many variables, such as viscosity, interface tension, micelle size, surfactant, the hydrophilic and lipophilic phase amounts and the physical nature between the components of the emulsion. At some critical mass, size and interactions between phases, the aggregates settle to the bottom of the container by gravitational forces. This can be accelerated by centrifugation, mechanical coalescence, or addition of one of the emulsion phases to change the superstructure (Lissant, 1983). Demulsification can occur by disrupting the integrity of the micelle superstructure and the thermodynamic properties of the emulsion. This can be done by temperature, mechanical means or chemical addition (Lissant, 1983). There are many systems designed to demulsify, but many are designed for industrial applications and are complex and expensive.

2.6.3 Sorption of Pesticides Waste from Liquid Media

Adsorption and absorption involve a complex set of physical and chemical interactions between particles. Adsorption differs from absorption in that adsorption involves the interaction of the solute molecule (sorbate) with the surface of the sorption matrix (sorbent), whereas absorption involves the penetration of the sorbate present in one phase (i.e. solvent) into another phase (i.e. solid sorbent) (Gregg and Sing, 1982). The term sorption can be used to denote the situation where both adsorption and absorption
processes are involved. Both of the sorption processes appear to occur particularly when the sorbent is solid in solution. Sorption processes can be defined as 3 major types: vapor phase, composite-liquid and solute. Vapor phase sorption involves the interaction of a gas or vapor with a solid surface and composite-liquid sorption involves the interaction of liquids with one another. Solute sorption involves the attractive interaction of solutes present in solution with a solid matrix (sorbent). For discussion the information provided here will focus on the solute sorption processes.

Sorption of solutes is influenced by the physical/chemical nature of the solute, solvent, sorbent or concerted interactions involving all components. Good sorption involves a strong affinity of the sorbent for the sorbate molecule.

Solvent factors such as solubility of the solute and pH influence the processes of sorption. How well a solute is sorbed depends on the lyophobic (solvent-disliking) characteristics of the solvent and the solute interactions. The tendency for sorption to occur increases as the lyophobicity of the solvent increases (Schweitzer, 1979). In other words, the solute may be more attracted to the sorbent phase than the solvent, depending on the solvent nature. For example, sorption of a hydrophobic solute molecule will increase if the solvent is made more hydrophilic. Conversely, the sorption of a given species will decrease if a solvent is chosen with higher solubility for the solute. A solute is considered to have amphoteric solubility if it has both hydrophobic and hydrophilic regions. One region may interact with the sorbent while the other remains associated with the solvent.

Molecules or atoms can be attracted to and become associated with a solid surface in either a physical, chemical or combined manner. In any case, sorption is governed by thermodynamics. The equation \( F = H - T \Delta S \) explains sorption processes, where \( F \) is the free energy (kcal), \( H \) is the enthalpy (energy content in kcal), \( T \) is the temperature (in °K), and \( \Delta S \) is the entropy of the system (kcal/°K). During most sorption processes, there is a decrease in entropy (S) because the solute molecules move from a three-dimensional region to a two-dimensional one, thus S is negative. Adsorption, in general, is exothermic for all gas and vapor phase sorptions and most solute-sorption processes (Giles and Easton, 1968).

Physical interactions of sorbent and solute, called physisorption, occur mainly by van der Waals forces which are comprised of both London dispersion forces and electrostatic interactions (Weber, 1985). These van der Waals attractions are long range weak interactions having energies in the same magnitude as the heat of condensation.
(under 100 kJ/mole) (Atkins, 1986). The sorbate that comes in contact with the sorbent will transfer energy as vibrational energy. The lattice of the sorbent absorbs the energy and the sorbate may become distorted due to the interaction with sorbent. The physisorbed sorbate vibrates in what is called a potential well within the sorbent. The potential well has a degree of attractiveness for the sorbate. If the energy involved with the sorbate vibrations is high enough, such as with increasing temperature, the sorbate may overcome the energy of the potential well and dissociate. The result of weak associations confers to an increased exchange rate. Conversely, the rate of exchange is decreased with lower temperatures.

Sorption of solute onto sorbent may involve chemical reactions and is called chemisorption. The interaction involves the formation of a chemical bond which usually is covalent. The exothermic energy of chemisorption is an order of magnitude higher (100-1000 kJ/mole) than those observed with physisorption. The rates of chemisorption depend on temperature and the activation energy of the reaction. If the activation energy is high, then chemisorption is fairly slow. Chemisorption exchange rates are usually slower than those observed with physisorption due to the stronger associations. Sorbent surfaces act as good catalysts for chemical reactions. Part of the product may remain bound to the sorbent after a reaction liberating other fragments into solution (Atkins, 1986).

Solute, solvent and sorbent affect the sorption and estimating or explaining the processes in a theoretical and quantitative manner is difficult even though each component can be categorized. The difficulty lies with the complicated interactions between the three phases. The quality of sorption is concerned with quantity of solute removed from solution by minimal amounts of sorbent. The ideal sorbent has a large surface area per amount of sorbent and a maximum amount of sorption sites. However, a good sorbent for one type of solute sorption may not be good for another type of solute due to the sorption site specificity. For example, one sorbent may sorb large amounts of hydrocarbons due to its hydrophobicity, but the sorbent may not sorb polar compounds.

To compare sorption qualities of various sorbate-sorbent systems, the adsorption isotherms are usually employed. The adsorption isotherm is an empirical method that compares the amount of sorbate sorbed to the mass of the sorbent and the amount of sorbate left in solution after an equilibrium has been achieved. The best isotherm would have a large sorption capability, low mass and virtually no sorbate left in solution at equilibrium. Different versions of the adsorption isotherm have been developed to explain
different adsorption systems and processes. The original Langmuir adsorption isotherm was developed to explain monolayer sorption of gases. The Brunauer, Emmett and Teller (BET) model was derived to explain polylayer sorption processes. However, both methods assume that the sorbent surface has homogeneous sites for sorption. The Freundlich adsorption isotherm was developed to explain sorption phenomena in solution with heterogeneous sites on the sorbent. The Freundlich equation is:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e$$

The $q_e$ is the amount sorbed/mass of sorbent, $C_e$ is the concentration of sorbate left in solution, $K_f$ is the relative indicator of sorption capacity and $1/n$ is indicative of the energy or intensity of the interaction (Weber, 1985). Other sorption isotherm models have been developed, but the Freundlich appears to best explain the sorption processes in solution (Weber, 1985).

Many sorbent types exist that remove various sorbates from solution. Certain sorbents are synthesized for use in chromatography that have ionic, hydrophobic or specific chemical sites for sorption. However, the majority of sorbents used in wastewater treatment are from naturally occurring sources (Schweitzer, 1979). Alumina, silica, clay and activated carbon are employed as sorbents for chromatography or waste removal, and each can sorb varying degrees of ionic, nonionic and hydrophobic solutes. Soil acts as a sorbent and, depending on the composition, such as the type of clay, can remove a wide variety of dissolved solutes from solution (Guenzi, 1975).

Organic-based materials, such as leaf litter, humus and peat moss, can also act as good sorbents due to exposed functional chemical sites. Peat moss possesses a significant cation exchange capacity and peat moss has been used to sorb organic pollutants from wastewater (Coupal and Lalanette, 1976; Rogozhkin, 1978). Mullins et al. (1989) have demonstrated the use of peat moss for a sorbent/substrate for pesticide biodegradation. Also, Best et al. (1972) have shown that peat moss is a good sorbent for removing cationic pesticides from solution. The pH of peat moss is fairly low (3-5) and at pH's above 6, there can be a breakdown of the matrix and substantial amounts of organic matter can become dissolved. Peat moss has been demonstrated to be a good sorbent for hydrophobic substances, such as oil (Mathavan and Viraraghavan, 1989). Heated peat moss can act as hydrophobic sorbent (Ekman and Asplund, 1975), sorbing up to eight times its weight in oil (D'Hennezel and Coupal, 1972). Peat moss and other related organic based sorbents have disadvantages for use as a sorbent (MacCarthy and Djobbar, 1986). Pigments and other organic matter can leach into solution which can present a
discolorization problem and act as site for dissolved pesticides to become sorbed and remain in solution.

2.7 Fate of Pesticides in Compost

Pesticides become associated with compost media in two basic ways: one by constructing the compost from yardwaste containing pesticides, and the other by purposely placing pesticides in the compost with the desired purpose to detoxify the pesticides through biological and physicochemical processes. Compost may be constructed entirely of yardwaste, as could be the case for the homeowner, or up to 18% yardwaste in municipal solid waste composting (Cantor, 1988).

Leemon and Pylypiw (1992) determined the fate of diazinon, chlorpyrifos, isofenfos, and pendimethalin in compost consisting of grass clippings that had the pesticides applied on lawns at the manufacturers' suggested rates. After 16 weeks in compost, all pesticides examined had decreased to below 0.01 µg/g from starting concentrations as high as 40, 16, 9, and 4 µg/g for pendimethalin, isofenfos, diazinon, and chlorpyrifos, respectively. In a similar study, a bioassay was performed to determine the phytotoxicity of mature compost containing 2,4-D, dicamba, and MCPP (Bugbee and Saraceno, 1994). The herbicides were applied to turf at application rates approximating actual use, which was considered to be 100%. Additional treatments included rates were 0.1, 0.4, 1, 4, 10, and 400%. One day after application, grass clippings were composted for a month followed by a two week curing stage. There was 100% tomato plant mortality in the 100% rate treatments of plants grown directly in compost or compost-amended soil. Some phytotoxicity occurred at the 0.4% application rate in plants grown directly in compost treatment, whereas, phytotoxicity was not apparent in the compost-amended soil until the 4% treatment. In this study, composting for one month left enough active parent compound in the potting media to be bioavailable and phytotoxic to tomatoes.

Berry et al. (1992) used enclosed bioreactors containing either peat moss or steam-explored wood fibers as the composting matrix to degrade atrazine and carbofuran. They reported that after 180 to 225 days, solvent-extractable atrazine and carbofuran from the biobased matrices decreased between 80 and 99.5% of initial concentrations between 700 and 7230 µg/g (mgpesticide/kg dry matrix). Hydroxyatrazine (2-hydroxy-4-ethylamino-6-
isopropylamino-1,3,5-s-triazine) and carbofuran-phenol (2,3-dihydro-2,2-dimethyl-7-hydroxy benzofuran) were the primary metabolites in the atrazine and carbofuran bioreactors, respectively. Bioassays were done involving either growing oat [Avena sativa L.] with atrazine or placing western corn rootworm larvae [Diabrotica virgifera virgifera (LeConte)] in carbofuran-treated compost matrix after the incubation period (276 to 480 days). In both cases, a contaminated peat moss and non-contaminated soil mixture at a 20:80 ratio (dry weight basis) was used as the potting matrix. No significant adverse effects were observed in the corn rootworm bioassays involving initial carbofuran concentrations of 700 to 5000 µg/g. In the wheat bioassays, only the wheat grown in the compost matrix with the initial atrazine concentration of 7230 µg/g was adversely affected.

In other studies involving a peat moss-based compost, diazinon concentrations mg pesticide/kg dry compost after 18 weeks decreased to between 7 and 1 µg/g from starting concentrations of between 4000 and 32000 µg/g (Mullins et al., 1989). The major path of diazinon degradation in the compost was determined to be hydrolysis to 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPH). Hydrolysis was probably accelerated due to compost temperatures (53 to 59 °C), and pH (2.7 to 5.1). The addition of ground corn meal and fertilizer influenced the biological activity as indicated by the heightened compost temperatures.

In a study involving carbaryl in sewage sludge composted for 20 days, between 2.6 and 4.2 % of the radioactivity orginally applied as naphyl-1-14C-carbaryl was recovered from compost extraction using organic solvents (Racke and Frink, 1989). The majority of the radioactivity (92-95%) was recovered by combustion of the unextractable fraction.

A study conducted by the Snell Environmental Group, Inc. (1982) involved determining the fate of several toxic compounds in compost. Inclusive in the list of compounds studied were several classes of insecticides and herbicides. They reported that trifluralin had a high susceptibility to degradation in compost (76-95% reduction after 30 days). Pesticides classified as susceptible to degradation at moderately high rates (51-75% reduction) were atrazine, lindane, endrin, and methoxychlor, and chlorpyrifos, chlorodane, and silvex (2-(2,4,5-trichlorophenoxoy)propionic acid were reported as having moderate susceptibility to degradation (31-50% reduction). The herbicide 2,4-D was found to have moderately low (16-30% reduction) susceptibility to degradation, and those pesticides found to be resistant to degradation in compost (low susceptibility = 0-15% reduction) were DDT, dieldrin, and toxaphene.
2.8 Factors Influencing the Fate of Pesticides

After a pesticide has been applied to control a particular pest, the parent pesticide molecules are subjected to a myriad of abiotic and biotic influences. The fate of pesticides in any environment or situation depends greatly on the pesticide’s physicochemical properties, as well as its location. Pesticides may be found in one or more of the three physical states of matter (i.e. gas, liquid, and solid) in locations or systems, such as lakes, rivers, streams, oceans, soil, soil water, sediment, groundwater, living organisms, the atmosphere, and inside places like the home or other human-made structures. As shown in Figure 2, the parent pesticide compound can be altered or exit a system by one or more of several pathways, which can include volatilization, photolysis, degradation, sorption processes, organismal metabolism, bioaccumulation, plant uptake, leaching, runoff, and other means of fate. Temperature, pH, oxidative/reductive state, mobility, bioactivity, and other influences also affect the rate at which pesticides can be altered or exchanged between one system to another, as well as the pesticide’s chemical and physical make-up. There are several good reviews and discussions on the fate pesticides in the environment (SSSA, 1974; Biggar and Seiber, 1987; SSSA, 1989; Samiullah, 1990; Schnoor, 1992; Mansour, 1993)

The fate of pesticides in the environment is complicated and there are many factors influencing the pathways of movement of molecules between phases and what happens to them in each phase (e.g. sorption and degradation). Some of the major influences are temperature, pH, sunlight, salinity, dissolved organic material, bioactivity, and the physicochemical properties of the pesticide molecule.

Equilibrium partitioning is the subdivision of a population of molecules between two phases (e.g. air and water). Over a period of time a population of molecules will move into a particular phase to greater or lesser extent, depending on the relative affinity that the molecules have for each phase. A water soluble compound has a stronger affinity for remaining in water than does a less soluble compound, therefore the less soluble compound will move to a more thermodynamically favorable situation. In some cases sorption to some solid substrate in the water or movement into the air may be the more thermodynamically favorable situation. Solubility in water of compounds depends largely on structure and chemistry of the compound and the physicochemical conditions of the surrounding aqueous environment. For the most part, the solubility of a compound is directly proportional to its polarity. The polarity of a compound comes about due to its
Figure 2 Processes of Pesticide Fate: Dissipation, Degradation, and Detoxification
structural asymmetry, charged or uncharged groups, and interactions with other surrounding molecules. The bonding angles of the hydrogen atoms in water in relation to the oxygen atom is such that negative and positive charges are conferred to the oxygen and hydrogen ends, respectively. The lack of charged groups and strong structural symmetry in a hexane molecule make it quite water insoluble. Intermolecular forces, or in other words, those interactions between molecules that are the same or different, also influence solubility and include polar interactions (dipole-dipole and dipole-induced dipole), hydrogen bonding, dispersion interactions, and van der Waals forces.

The degree to which a pesticide will leave a system by volatilization is indicated by the vapor pressure of that pesticide, as well as the temperature, solubility characteristics, air-water partitioning (Henry's law coefficients), and the type and amount of exposure to the atmosphere. Volatilization occurs when condensed molecules acquire energy to overcome forces of attraction to adjacent molecules in their present situation or system and enter the vapor phase, which increases the entropy of the system. The more attractive a molecule is to similar or different molecules, the more energy it takes to volatilize the molecule. The polarity of a molecule, represented by the value for the dipole moment, influences how much attraction molecules will exhibit with one another. The classic case is the decreasing vapor pressure of benzene-containing compounds with increasingly more polar moieties attached. Also, molecules of lower molecular weight within a particular class of chemicals will have higher vapor pressures. The magnitude of attraction between molecules within a similar class is greater for increasing larger molecules due to the cumulative effects of the van der Waals forces. Therefore, the larger the molecule, the larger the magnitude of van der Waals attraction between molecules, and consequently greater vapor pressures. Considering the various parameters that describe compounds, such as the vapor pressure, aqueous solubility, molecular weight, structure, polarity, etc., it appears that Henry's law constant is the best parameter for predicting the volatilization rate (Jury et al., 1983).

Pesticide photolysis can occur in air, water, or on solid surfaces, and is directly proportional to the amount of light energy the molecules are exposed to and how much of that energy is absorbed. Due to the nature of a molecule's makeup, some molecules absorb more light energy at one or more wavelengths. The absorbed energy creates a situation of instability within the molecule causing the molecule to dissociate in some fashion that relieves the structural stress. In addition to the direct effect of sunlight on compounds, there are indirect effects caused by the formation of other compounds that
come about from sunlight-mediated processes, such as the unstable radical form of oxygen called singlet oxygen. The formation of singlet oxygen occurs readily in the atmosphere. Singlet oxygen is quite reactive and its interaction with many organic compounds, such as pesticides, results in degradation.

Degradation of pesticides can be mediated by abiotic or biotic processes, and various combinations of each. Some of the abiotic pathways of degradation that occur are hydrolysis, oxidation, and reduction. Hydrolysis is a common abiotic degradative pathway, which involves the nucleophilic attack on the pesticide molecule by water molecules. The predominant type of pesticide hydrolysis involves the $\text{Sn}_2$-type of reaction where the nucleophilic attack occurs on the opposite side of a leaving group, and an intermediate is formed. The nature of the compound and environmental conditions (e.g. pH, redox potential, oxygen, temperature) are important factors for determining the extent and rate of hydrolysis. Chemical degradation of pesticides can also occur by sorption-catalyzed reactions (Armstrong and Chesters, 1968) or by interactions with soil organic matter (Armstrong and Conrad, 1974), as well as photolysis.

In addition to abiotic degradation, many pesticides are degraded externally or internally to microorganisms, plants, and animals that use the pesticide molecule as an energy source or part of catabolism (Kearney and Karns, 1987). The common biotic reactions that occur are classified as phase I and phase II type of metabolism. Examples of phase I metabolism are oxidative, reductive, and hydrolytic processes. These phase I reactions are catalyzed by enzymes and may not occur abiotically to any great extent because the reaction is not thermodynamically favorable. Phase II metabolism also involves enzymes and the result is conjugation with compounds like carbohydrates, sulfates, alcohols, peptides, and amino acids.

Microorganisms are ubiquitous, living in all earthly environments having varying concentrations of oxygen. There are regions in the soil profile, groundwater, and submerged soils that are predominantly anoxic, supporting a variety of anaerobic microorganisms (Young et al., 1984). Other regions in the soil closer to the soil-atmosphere interface have oxygen concentrations great enough to support the aerobic bacteria. It is these aerobic soil microorganisms that predominate in well aerated compost (Biddlestone, et al., 1987).
2.9 Fate of Atrazine

Atrazine is a photosynthetic inhibiting herbicide selective for annual grasses and broadleaved weeds. Triazines are primarily soil applied herbicides that are absorbed through the root system, but can be applied foliarly if surfactants are present. Atrazine inhibits photosynthesis in the "Hill reaction" or photosystem II. It has been suggested that atrazine, being fairly lipophilic, binds to the plastoquinone site in the photosystem II path, thus disrupting proper exchange of electrons. It is believed that free radicals form as the flow of electrons in the photosynthetic pathway are disrupted. The free radicals formed interact with plant tissue, which results in cellular damage and eventual plant death.

The common field rate for atrazine was reduced to 3.3 kg a.i./ha in 1990 from an average range of 2.2 to 4.4 kg a.i./ha, and reduced again in 1993 for highly erosive land to 2.7 and 2.2 kg a.i./ha with >30% and <30% residue coverage, respectively (Steinheimer, 1993).

Atrazine is fairly lipophilic, having a water solubility of 33 μg/mL. Atrazine has a vapor pressure of 0.037 mPa (1.4 X 10⁻⁶ mm Hg), therefore volatilization is not a major route of dissipation of atrazine (Howard, 1991).

The half-life or DT₅₀ of atrazine in soil is quite variable depending on soil type, soil organic matter, soil pH, and microbial activity. DT₅₀ values of atrazine dissipation have been reported to be between 14 days in a soil microcosm (Winklentian and Klaine, 1991a) to 400 days in autoclaved soil (Qiao, et al., 1994). Bacci et al. (1989) reported an atrazine DT₅₀ of approximately 50 and 168 days in field soil and in natural lake waters. Qiao et al. (1994) report atrazine DT₅₀ values of 65-70 days in fallow fields and 150-160 days in cultivated fields. Kruger et al. (1993) reported an atrazine DT₅₀ range from 41 to 231 days in Iowa loam and sandy clay loam soils. In addition to soil characteristics having a large role in atrazine dissipation, the climate or relative temperature also is important. Frank et al. (1991) demonstrated that atrazine applied to clay loam soil in a Canadian field during the summer months had DT₅₀ values ranging from 37 to 64 days, but increased to 125 days during the winter. Application of atrazine before the winter resulted in a DT₅₀ of approximately 198 days.

The fate of atrazine appears to follow two major routes. One route of atrazine dissipation in the environment is a combination of abiotically and biotically mediated degradation with mineralization as a possible result. The other dissipation route involves bound residue formation of atrazine, its metabolites, and the organic matter. Atrazine in
soil is continually degraded by abiotically and biotically mediated reactions. The resulting metabolites can be further degraded through biologically mediated reactions. Microbial mineralization of atrazine to carbon dioxide, which is the complete destruction of the original molecule, may occur depending on the microbial activity, the species profile present, and whether or not bound residue formation occurs.

The second route effectively ends with atrazine or its metabolites becoming bound to the organic matter in the soil. Once chemically bound, the identity of the original molecule is essentially removed (Berry and Boyd, 1985). The original molecule, atrazine or one of its metabolites, can become unbound through degradation of the organic matter, but this process is slow, and at any one time, the amount freed is considerably low. Eventually, there is a complete turnover of the organic matter input into a soil system back to carbon dioxide. The complete mineralization process of vegetative material entering the soil organic matter to carbon dioxide is constantly occurring, but slow. The mean residence time ranges between 250 years to above 900 years, depending on the climate, soil, and vegetative input (Stevenson, 1982).

Sorption of triazines to soil colloids and soil organic matter does occur to a considerable extent, therefore sorption is a major factor in the environmental fate of atrazine. Increasing amounts of soil organic matter, clay content, temperature, and decreasing pH have been shown to increase triazine sorption in soil (Talbert and Fletchall, 1965). Dao and Lavy (1978) determined that decreasing the moisture content of the soil increased atrazine sorption, as indicated by the Freundlich adsorption isotherm coefficient ($k_F$). Clay content and clay type influence atrazine adsorption. Atrazine is a weakly basic compound with a $pK_a$ of 1.7 (Hance, 1971). As soil conditions become more acidic, an increasing proportion of atrazine molecules become protonated and can associate with negatively charged clay surfaces. Even though positive correlations have been demonstrated between the extent of clay in soil and clay types with atrazine adsorption, there has been considerable variability, which may be due, in part, to the low $pK_a$ of atrazine and the relative amount of cation exchange with clay, which depends on the soil pH (Weber, 1970). Therefore, a general rule of correlating clay content in soil with atrazine adsorption should not be followed (Dousset et al., 1994). It has also been demonstrated that complex formation with bi- or multivalent cations (i.e. Ca$^{2+}$, Fe$^{3+}$, Ce$^{4+}$) does not appear to be a predominant adsorption mechanism for atrazine (Hance, 1971). However, the largest influence on atrazine adsorption appears to be the soil organic matter content (Dousset et al., 1994) A strong correlation exists between the
extent of sorption of non-ionic compounds with decreasing water solubility and increasing soil organic matter content (Hamaker and Thompson, 1972). The degree to which organic matter has matured or humified has also been shown to influence atrazine adsorption (Paya-Perez et al., 1992; Dousset et al., 1994).

The adsorption of atrazine is a reversible process, and is thought to occur in two distinct phases (Karickhoff and Morris, 1985; Pignatello, 1989). These two phases include an initial rapid equilibrium component, followed by a slower phase that involves the infiltration of the sorbate (atrazine) into the organic matrix and sorption. The rapid phase adsorption is reversible, thus considered to be labile for microbial attack, however, the slower phase is considered to be only slightly reversible, and as consequence, resistant to microbial attack (Karickhoff and Morris, 1985; Pignatello, 1990a, 1990b). Pignatello and Huang (1991) demonstrated that atrazine adsorption becomes less reversible as residues age. They suggested that diffusion of atrazine into the matrix and the increase in stronger binding over time decreases the movement of atrazine back into the bulk water phase.

In a widely referenced article (Armstrong and Chesters, 1968), it was reported that abiotic hydrolysis of atrazine to hydroxyatrazine was mediated or catalyzed by adsorption of atrazine. More specifically, the hydrolysis resulted from hydrogen bonding of the atrazine ring nitrogen and the carboxy group of the organic phase in soil or artificial carboxyl resin. The ring carbon associated with the chlorine atom is electron deficient due to the electronegative chlorine and ring nitrogen atoms. The electron deficient ring carbon is susceptible to abiotic and biotic nucleophilic attack or hydrolysis. Hydrogen bonding of the ring-nitrogen hydrogens increases the electron deficiency of the ring carbon, therefore increasing the susceptibility to hydrolysis.

Hydrolysis of atrazine under field conditions is catalyzed by soil components, such as soil organic matter (i.e. humic substances) and soil colloids (Gamble and Khan, 1985, 1988, 1990). Gamble and Kahn in the aforementioned articles reported that the mechanism of atrazine hydrolysis catalyzed by humic substances involves the acidic functional groups of humic and fulvic acids (i.e. free carboxyl groups). As atrazine becomes sorbed to the soil organic matter, the association of the acidic groups and the slightly positive ring-nitrogen in atrazine increases the susceptibility of atrazine hydrolysis.

Hydrolysis of atrazine to hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine) has been reported to be the major degradative process (Skipper et al., 1967; Benyon et al., 1972; Kaufman, 1974; Muir and Baker, 1978; Kahn and
Saidak, 1981; Jones et al., 1982; Winkleman and Klaine, 1991a). Phototoxicity appears to be eliminated with the formation of hydroxyatrazine (HYA) (Kaufman and Kearney, 1970). The hydrolysis of atrazine to hydroxyatrazine is thought to be a predominately abiotic process (Armstrong et al., 1967). Several researchers have shown that the formation of hydroxyatrazine occurred similarly in microbially active soil and sterile soil, which suggested to them that the hydrolysis process is abiotic (Skipper et al., 1967; Winkleman and Klaine, 1991a; Demon et al., 1994; Qiao et al., 1994). As an example, Winkleman and Klaine (1991a) found HYA in sterilized soil microcosms that was approximately 76% of the original amount of atrazine applied at a rate of 5.6 μg/g. They found HYA at 41% of the original amount of atrazine applied in the same soil, which was biologically active. The dissipation of atrazine was similar between biologically active soils and sterile soils, and they attribute the difference in HYA concentrations between the soil to greater biologically mediated metabolism of HYA formed in the biologically active soil. Qiao et al. (1994) demonstrated that hydrolysis of atrazine to HYA occurred as readily in autoclaved soil as in biologically active soil.

On the other hand, there are studies that have shown that the hydrolytic process can be correlated with increases in microbial activity. Harris (1965) found that the hydrolysis of atrazine was accelerated in microbially active soil compared to less active soils and sterilized soil. Mandelbaum et al. (1993) have shown that atrazine hydrolysis can be mediated by microbial metabolism. They used bacterial isolates from over 100 different atrazine-degrading soils and proved that the atrazine hydrolysis they were observing was enzymatically-mediated. They suggest that in previous studies that sterilized soil by heating, may have actually selected for heat tolerant bacteria, which may have been more able to degrade atrazine. In any case, the role of microbially mediated hydrolysis of atrazine may be more important than previously suspected.

In addition to hydrolysis, dealkylation of atrazine and hydroxyatrazine metabolites also have been shown to occur in soil (Muir and Baker, 1978; Kruger et al., 1993; Winkleman and Klaine, 1991a; Adams and Thurman, 1991). N-dealkylation appears to be another major pathway of atrazine degradation and has been shown to occur through microbially mediated reactions (Kaufman and Kearney, 1970). The two possible chlorinated mono-dealkylated metabolites, de-ethylatrazine and deisopropyl atrazine, retain a phytotoxic effect - de-ethylatrazine is nearly as phytotoxic as atrazine and deisopropyl atrazine is five times less phytotoxic (Kaufman and Kearney, 1970). However, the totally dealkylated metabolite, diamino-atrazine, does not appear to retain a
phytotoxic effect. The formation of de-ethylatrazine (DEA) in soil has been shown to be the more likely than the degradation of atrazine to deisopropylatrazine (DIA) (Skipper and Volk, 1972). Kruger et al. (1993) found that DIA degrades under saturated soil conditions quicker than atrazine, and therefore, may explain why DEI is not found in groundwater to the extent that atrazine and DEA are found.

Further biological degradation of atrazine and its metabolites continues to occur until bound residue formation or complete mineralization occurs. The natural degradation course of non-polar compounds is for them to become increasingly more polar until they are completely mineralized or become bound to the soil organic matter. Apparently, as microbial attack on the compound occurs through enzymatically mediated reactions, which allows microbes to sequester carbon and energy from the compound, the resulting product is more polar. For microbial populations metabolizing an aromatic structure, polar constituents are formed or added enzymatically to the ring structure, thus decreasing ring stability, increasing polarity, and allowing the aromatic ring structure to be disrupted by further enzymatic attack (Gibson and Subramanian, 1982). This pattern is observed with atrazine dissipation in soil (Dao et al., 1979; Baluch et al., 1993). Complete mineralization of atrazine has been demonstrated to occur in soil by pseudomonads (Jesse et al., 1983; Cook et al., 1985). Mineralization of atrazine applied to soil at field rates (5.4 µg/g) accounted for 12 to 28% of the original amount of atrazine applied at normal field rates of 5.4 µg/g after 180 days (Winkleman and Klaine, 1991a). Nair and Schnoor (1994) reported that between 3 and 15% atrazine mineralization at field rates, and attributed the degree of mineralization to soil variables, such as soil type, organic matter content, oxygen content, and soil moisture. The mineralization rates were directly proportional to the organic matter content, and the biological degradation was limited by the oxygen content of the soil atmosphere. At the other extreme, Goswami and Green (1971) reported only 0.005% mineralization after 12 weeks, however, the study used submerged soils which may have not supported aerobic microbial populations. Skipper et al. (1967) reported less than 3% mineralization of atrazine after 2 weeks in soil under aerobic conditions. They do not suggest a reason for low mineralization. Less than 1% of the atrazine originally applied to soil mineralized after one month in a later study by Skipper and Volk (1972). Low mineralization of atrazine in earlier studies compared the higher mineralization observed in recent studies appears to be the norm, which may be due in part to using more active soils and taking measures to optimize the conditions for microbial growth.
The fate of hydroxyatrazine (HYA) and the other atrazine degradates in soil is different than the fate of atrazine. In several studies it has been shown that the hydrolysis of atrazine to HYA is more rapid than the subsequent degradation of HYA (Goswami and Green, 1971; Skipper and Volk, 1972). Winkleman and Klaine (1991b) reported that the degradation of HYA, indicated by half-life, was slower than deisopropylatrazine (DIA), de-ethylatrazine (DEA), and didealkylatrazine (DAA) under similar conditions. It has been shown that HYA is more strongly bound to the soil and soil organic matter than atrazine (Stevenson, 1972; Clay et al., 1988; Mandelbaum et al, 1993). The binding or sorption of HYA to the soil and soil organic matter appears to be mainly attractive, and therefore reversible, since HYA can be effectively recovered during extraction with organic solvents. It has been shown that HYA is least likely to form bound residues with the soil organic matter (Schiavon, 1988a, Winkleman and Klaine, 1991b). It may be that as HYA becomes quickly and strongly sorbed to the organic matter in soil, the process of bound residue formation is minimized. The primary constituent of the atrazine molecule is most likely an exposed amino group (either the isopropyl or ethyl side-chain). Winkleman and Klaine (1991b) showed that didealkylatrazine (DAA) forms the greatest amount of bound residue in sterile soil microcosms, followed by the mono-dealkylated forms of atrazine, and finally HYA. Conversely, in the same study, DAA was mineralized at the greatest rates in similar soils that were biologically active. These data suggest that bound residue may be microbially facilitated by dealkylation, thus exposing the amino groups, but the actual mechanism of bound residue formation may occur abiotically.

2.10 Fate of Chlorpyrifos

Chlorpyrifos or O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate is formulated mainly as an emulsifiable concentrate and is one of most widely used broad-spectrum organophosphorus insecticides (Cink and Coats, 1993). Chlorpyrifos is used for controlling pests in wide variety of situations, such as agriculture, horticulture, turf management, pets, and home and human structures.

Studies have demonstrated that chlorpyrifos applied at normal rates (approximately 10 μg/g dry soil) dissipates in actual field situations between 3 and 4 months after application in mineral and organic soils (Chapman and Harris, 1980). Racke et al., (1993) reported that chlorpyrifos applied topically to soil at 5 to 10 μg/g had DT50 values
between 1 and 17 days, whereas DT$_{50}$ values of chlorpyrifos incorporated into soil were between 33 and 56 days. DowElanco report an average chlorpyrifos soil dissipation DT$_{50}$ in their technical data sheet of 68 $\pm$ 13 days in 10 different soils at 25 °C DowElanco, 1989). On the other extreme, DT$_{50}$ values have been reported to be between 116 and 1576 days for chlorpyrifos applied to soil at rates of 1000 µg/g for termite control (Racke et al., 1993; Racke et al., 1994).

Chlorpyrifos dissipation can occur by volatilization (Harris and Hitchon, 1970) and by abiotic and biotic pathways (Miles and Harris, 1979). It has a vapor pressure of 1.88 X 10$^{-5}$ mm Hg or 2.53 mPa, making it a somewhat volatile insecticide. The amount of volatilization can be affected by several parameters, such as the depth of application, amount of air turbulence (wind), temperature, soil moisture, soil organic matter content, and biological activity (Spencer, 1987).

Chlorpyrifos dissipation in soil and aquatic environments appears to occur by hydrolytic pathways rather than biodegradation (Macalady and Wolfe, 1983). They demonstrated that the hydrolysis of chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP) is a pH dependent reaction and follows first-order kinetics at pH's of 4-7.5. At pH's above 8, alkaline hydrolysis predominates, but the kinetics do not appear to be second-order as expected from alkaline hydrolytic processes even though an anionic intermediate is formed. Additionally, the methoxy derivative of TCP called 2-methoxy-3,5,6-trichloro-2-pyridinol has been found in soil samples and its presence has been attributed to microbial transformation (Csinos, 1985). Chapman and Harris (1980) discovered traces (0.001 µg/g) of oxyclochlorpyrifos in organic and mineral soil samples taken immediately after treatment. Oxychlorpyrifos did not appear to be stable because concentrations decreased soon after treatment. They reported that chemical hydrolysis appeared to be the major degradative pathway with TCP being the predominant end product. The presence of cations in solution or soil appear to enhance the hydrolysis of chlorpyrifos (Blanchet and St-George, 1982). They reported that cations, such as copper and calcium, formed intermediates with chlorpyrifos which increased the rates of degradation.

The role of microorganisms in mediating chlorpyrifos degradation is questionable. Evidence supports that chlorpyrifos degradation is increased by the presence of soil microorganisms (Getzin and Rosefield, 1968; Miles et al. 1983). The influence of microbial activity has been demonstrated for other organophosphates, such as diazinon (Forrest et al., 1981), isofenfos (Racke and Coats, 1987), fensulfothion (Read, 1983), and terbufos (Harris et al., 1988). However, Racke et al. (1993) also demonstrated that
chlorpyrifos degradation did not increase in several studies using methods for enhancing microbial degradation, such as pretreatment with chlorpyrifos, using soils with a chlorpyrifos history, or increasing bioactivity.

The seemingly contradictory statements made about the role of microorganisms may be explained by Racke et al. (1988), stating "that although soil microorganisms do play an important role in chlorpyrifos degradation, soil microbes are generally unable to beneficially catabolize chlorpyrifos." Racke et al. (1990) explained that there are four basic reasons why beneficial catabolism or enhanced degradation may not occur with certain pesticides. These reasons include the stearic hindrance of soil enzymes (Burns and Audus, 1970; Niemi et al., 1987), the prevention of biodegradation by sorption characteristics (Ogram, et al., 1985), the inability for microbial consortium to degrade the pesticide beyond a certain metabolite (Tiedje and Hagedorn, 1975), and the inability for microbial populations/soil enzymes to degrade pesticides/metabolites under certain conditions, such as pH. Another reason may involve inhibition of microbial metabolism. It has been demonstrated that TCP is toxic to soil microorganisms, and, therefore, is recalcitrant to further degradation (Somasundaram et al., 1990, Racke et al., 1988). Several studies indicated that as the soil concentration of chlorpyrifos is increased, the rate of chlorpyrifos degradation decreases (Cink and Coats, 1993; Racke et al., 1993, and 1988; Racke et al., 1994). The observed decrease in the rate of chlorpyrifos degradation may have been a result of TCP toxicity to soil microorganisms, since TCP accumulated as more chlorpyrifos degraded by abiotic and microbially mediated reactions. Mineralization of TCP to CO₂ did not occur in several sterile soils that had occurred when these soil were viable, implicating the role of microorganisms (Racke et al., 1988). In the same study, 80% of TCP applied to soil at 5 μg/g was mineralized after 2 weeks, whereas only 4% of TCP applied at 50 μg/g was mineralized after 2 weeks in the same soil under the same conditions. In fact, Racke et al. (1988) showed that degradation of soil-applied TCP at 100 μg/g was almost completely inhibited. It appears that chlorpyrifos will degrade to TCP by abiotic and microbially mediated reactions, but when the TCP concentration in soil is high enough, the role of microorganisms becomes inhibited, and further degradation occurs primarily by abiotic reactions.
2.11 Composting and Factors Influencing Compost Productivity

As previously mentioned, composting is an important method for the disposal of chemical wastes including pesticides. The nature of composting is defined by several researchers as being biological metabolism of organic wastes produced by human activities in a contained situation where conditions can be manipulated (Golueke, 1972; Polprasert, 1989). Because composting is governed by biological systems, those factors influencing living organisms are important to consider when augmenting the compost macrocosm. Golueke (1972) outlined these constraints as: 1) specific microbial populations must be present; 2) the rate of organic material degradation is directly proportional to the rate of microbial activity; 3) the compost size and capacity is governed by the microbial population present; 4) the compost matrix must be or have organic material for a microbial energy source; 5) environmental factors are the determining factor for the success and rate of biodegradation.

Since the majority of biodegradation in compost occurs by microbial activity (Polprasert, 1989), some researchers make a differentiation between anaerobic and aerobic composting (Pereira-Neta, 1987). The majority of composting deals with aerobic conditions and Biddlestone et al. (1987) suggested that organic material degradation occurs at faster rates than under anaerobic conditions. There is a succession of microbial populations that occur in aerobic composting and these include both mesophilic and thermophilic activities leading to the mineralization and humification of organic material present (Pereira-Neta, 1987). Bacteria and fungi constitute the majority of organisms present in the compost and those responsible for organic material degradation (Biddlestone et al. 1987). According to Polprasert (1989), primary degradation occurs by mesophilic bacteria, fungi and actinomycetes. Further biodegradation and mineralization is carried out first by mesophilic and then by thermophilic bacteria as compost temperatures rise above 45 °C. Mesophilic bacteria are present in the compost succession, followed by thermophilic bacteria. At bacterial-produced temperatures above 65 °C, most fungi, actinomycetes and most bacteria become inactive, and only spore-forming bacteria can develop (Storm, 1985). After the thermophilic and mesophilic populations have decreased due to the lower amount of organic matter, the fungi and actinomycetes become important in further biodegradation (Storm, 1985).

All levels of biological succession in the compost environment are influenced by the amount of energy present and physical and chemical factors. The important energy
requirements are the basic organic nutrients and the carbon and nitrogen ratio. Physical and chemical factors include oxygen concentrations, moisture, pH, and temperature.

2.11.1 Energy Sources

Microbial populations require an energy source which can be carbon, nitrogen or sulfur. However, in composting with aerobic conditions the important energy source appears to be carbon based. Simple and complex carbohydrates, lipids, proteins and other carbon based compounds found in nature can be used by growing microbial populations. When these energy containing compounds are mineralized, microbial populations decline. Certain nutrients are utilized first leaving the more metabolically difficult compounds, such as cellulose, lignocellulosic materials, and certain aromatic compounds, for fungi, actinomycetes and bacteria to utilize. In general, microbial populations will use the energy source that they can metabolize in the most efficient manner. One species may be able to utilize a specific chemical, whereas another species might not be able to metabolize the same compound.

2.11.2 Nutrients

Other important factors involved with the successful growth of microbial populations involve carbon to nitrogen and phosphorus ratios. Also, other minerals are needed by living organisms for proper growth and enzyme activity. Calcium, iron, potassium, sulfur and other micronutrients may or may not be required by microorganisms. Most living organisms need nitrogen and phosphorus for growth and enzyme function, as well as many other elements. Without an adequate nitrogen source, microbial populations within compost do not increase above a certain population (Golueke, 1972). A carbon to nitrogen ratio of 25:1 appears to be optimal (Golueke, 1972; Biddlestone et al., 1987). Polprasert (1989) suggested that a range of 20 to 40 (C/N) should serve as an adequate amount of nitrogen for most composting situations. The C/N ratio will increase if nitrogen is not added during the course of the compost succession. Haug (1980) reported a C/N ratio of 20 for the first 12 days in a compost followed by 20-50 and 78 after 14 days and 21 days, respectively. C/N ratios outside the
optimal 20-40 ratio appear to have detrimental effects on microbial populations and depends on the microbial population present (Fog, 1988).

2.11.3 Oxygen Requirements

The availability of oxygen within the compost matrix is important to ensure optimal microbial growth and metabolism. The oxygen is used for catabolic activity and for those oxidative reactions involved with substrate biodegradation (Polprasert, 1989). Oxygen demand is required throughout the succession, but the demand is greatest when thermophilic organisms are present (Biddlestone et al., 1987). Biddlestone et al. (1987) recommended that the oxygen concentration should remain between 10 and 18% for maximum thermophilic activity, which is achievable in forced-air composting. Mathur (1991) reported that the long standing "rule-of-thumb" for minimum oxygen content in compost is 5%. However, as in soil, there is a continuum of oxygen concentration in various sites, which allows a range of microaerophiles, obligate anaerobes, and strict aerobes to grow as oxygen conditions permit (Tate, 1995). Depletion of oxygen in these microsites primarily occurs because of reduced oxygen tension in water and that oxygen may be used for respiration, lowering the concentration to anaerobic or near anaerobic conditions. Thorstrup (1985) from several experiments suggested that oxygen content should not fall below 10%, even though 7% is not limiting. Wiley and Pearce (1955) recommended oxygen rates to be at least 6-19 mg hr\(^{-1}\) g\(^{-1}\).

To maintain the optimal oxygen content and turnover, the compost should have adequate air spaces in the matrix for aeration. The heat produced by the microbial population should assist in oxygen turnover, but some compost containments have mechanized air blowers installed (Polprasert, 1989). Vegetative material and bulking material, such as bark chips, are usually added to composting containments designed to facilitate oxygen flow through the matrix. Above ground compost windrows are popular because they are easily managed and a large amount of surface area is exposed to the atmosphere (Golueke, 1972; Biddlestone et al., 1987; Polprasert, 1989). Other compost designs have blowers and/or vents for aeration as part of the construction (Polprasert, 1989).
2.11.4 Moisture

Another important factor in successful compost activity involves moisture content. Actual moisture levels can exceed 100%, depending on the material. However, in practice moisture contents above 80-85% cause a decrease in available oxygen due to the limited air space filled by water or collapsed matrix (Golueke, 1972). Matrix water decreases the flow of air and oxygen is rapidly depleted. Anaerobic metabolism, rather than aerobic metabolism, will prevail in compost that is too hydrated. The amount of moisture required depends on the type of compost matrix. Manure, lawn clippings and paper are examples of matrices that need to have a moisture content between 50 and 65% (Golueke, 1972). Straw and wood products can hold more water (75-85%) due to their bulk matrix and resulting air spaces (Golueke, 1972). However, peat moss based composts are generally hydrated at 70 to 90% water/wet matrix (w/w) because of its water holding capacity (Mathur, 1991). Apparently, the additional water does not decrease air flow in the peat moss matrix. Peat moss on weight per weight basis is a better absorbent material that equal weights of common compost materials, such as grass clipping, straw, and leaves. Peat moss can hold up to 20 times its dry weight in water (Bulganina et al., 1983).

2.11.5 pH and Temperature

Each group of microbes has its optimal temperature range. Mesophilic bacteria function best at temperatures between 25-45 °C, and if conditions are adequate, thermophilic bacteria create a 55-65 °C optimal environment (Polprasert, 1989). There does not appear to be a clear homogeneous pattern of temperatures within a compost or throughout time (Golueke, 1972). Various microorganisms can thrive at various points within the compost over time because of insulating effects or exposure to the perimeter of the compost. The design and size of the compost is important to facilitate one temperature range or the other. A larger volume will facilitate thermophilic behavior, but aeration becomes important as the compost gets larger. In any case, the temperature is reflective of microbial populations and compost design and structure.

The optimal pH range for most bacteria is between 6 and 7.5 and between 5.5 and 8.0 for fungi (Golueke, 1972). In general, bacteria cannot tolerate a large deviation from their optimal pH range, therefore it is important to change or maintain the pH around
neutral conditions (Golueke, 1972). During the composting process, a small pH decrease may occur because of acid formation from microbial metabolism. The amount of acid formed should disappear because of mineralization (Polprasert, 1989).

2.11.6 Compost Biota

Composting in its very nature is a highly biotic process of vegetative decomposition. Decomposition of dead vegetative material occurs in all environments through a combination of microbial and invertebrate metabolism. If vegetative material is heaped to form a compost, decomposition increases many times the normal rates. This happens primarily because bacteria are able to reproduce rapidly using the easily metabolized compounds, such as proteins, amino acids, lipids, carbohydrates, and polysaccharides, such as chitins and pectins, in newly dead vegetation. Figure 3 illustrates the food web in compost with bacteria, actinomycetes, and fungi being primary consumers. Bacteria are the predominant group in the compost, especially at the beginning of the composting process. Common genera of bacteria found in compost include *Bacillus*, *Clostridium*, and *Pseudomonas* (Miller, 1991). Mesophilic bacteria and fungi rapidly grow and compete for the easily metabolized compounds. Their metabolic activity releases heat to the surrounding compost, and as the compost temperatures rise above 45 to 50 °C, the mesophilic microorganisms begin to die and form spores because they cannot survive at higher temperatures. At temperatures above 45 to 50 °C, thermophilic bacteria become predominant and are almost the exclusive group in the compost at higher temperatures. If there is a large enough volume of vegetation acting as insulation, internal compost temperatures can get as high as 77 °C (Martin and Gershuny, 1992). As the energy sources are used, the thermophilic bacterial populations decrease, and the compost temperature decreases. The largest biomass may have been present during the thermophilic stage, but species diversity becomes greatest as the compost cools (Polprasert, 1989). Fungi and actinomycetes are able to grow, and they have the ability to metabolize the more difficult compounds to metabolize, such as lignocellulosic material. As the compost temperature decreases below 40 to 45 °C, invertebrates move into the compost. Springtails, mites, nematodes, flatworms, and other insect larvae feed on bacteria, fungi, and actinomycetes, and it is their physical activity or diminution that actually increases decomposition (Siepel and Maaskamp, 1994; Vikram et al., 1994).
Figure 3

The Compost Food Web

Modified from Daniel L. Dindal, Ecology of Compost: A Public Involvement Project
The diminution of vegetation creates a larger surface area for microorganisms to grow and feed, which, in turn, provides more food for the secondary consumers (i.e. invertebrates). There are tertiary consumers (predators) that include predaceous beetles, mites, ants, centipedes. The ecological succession in compost is interesting, and it appears that all species involved are important in decomposing vegetation in the compost.

2.11.7 Microbial Activity and Pesticide Effect on Microbial Activity

Most composting situations involve fresh organic material, which acts as a large energy source for compost biota. The populations of a variety of organisms in compost grow, reproduce, interact with other species in the compost ecosystem, and use the energy source that comprises the compost matrix for metabolic processes. At times the cumulative metabolism of many compost organisms can be quite high, but then as the readily available energy sources in the compost are depleted, populations decrease and overall metabolism decreases.

The rate of biological activity at any one time in compost involves metabolic parameters. During metabolic processes, heat is released. Under aerobic conditions, metabolizing organisms consume oxygen and release carbon dioxide. The metabolic processes become a little more complex as the oxygen concentration decreases and other compounds become electron acceptors during respiration. In many cases, methane, hydrogen sulfide, ethanol, butyric acid, and other reduced molecules can be released as the by-product of electron transfer under oxygen-limiting conditions.

Anthropogenic compounds introduced into the environment can have an inhibitory or stimulatory effects on the metabolism and growth of microflora (Somerville et al., 1987). Because pesticides are designed to disrupt some metabolic pathway in a target pest, be it fungus, plant, insect, or rodent, several pesticides and some of their metabolites can be toxic to microbial metabolism. If an inhibitory effect on microbial activity is observed, the effect may be reversible or the effect may linger and essentially be irreversible over a period of time. A reversible situation may involve one in which the toxicant inhibiting overall microbial activity may dissipate through abiotic means or even may be biologically mediated. If one microbial species or a small consortium can degrade toxicants, their levels will eventually drop to the point where the affected populations can
resume normal metabolism. Also, affected populations of microorganisms may be able to adapt so that they metabolize the toxicant. A period of microbial adaptation is generally called a lag phase. However, if the pesticides or by-products are not toxic to microbial metabolism, they may actually stimulate microbial activity and growth due to the new influx of a carbon and/or nitrogen source. Elmholdt (1992) reports that when the fungicide propiconazole is applied at and below agricultural rates, the microbial activity is stimulated. Since a nutrient amendment (Lucerne medium) was added to all treatments to standardize the test and prevent any limitation by carbon/nitrogen flushing with fungicide addition, the stimulation was believed to be caused by a sub-toxic stress effect. The theory of a sub-toxic effect, proposed by Killham (1985), creates a stressful situation for those microbes affected, which results in diverting microbial metabolism from biosynthesis to maintenance energy requirements. Elmholdt (1992) also reported that propiconazole at unrealistically high rates results in a period of inhibition followed by recovery within 30 days, and then an increase of microbial activity compared to controls. She believed that after an initial inhibitory period, the additional carbon supplied by the accelerated rate of propiconazole stimulates additional microbial activity and growth compared to controls.

Martinez-Toledo et al. (1992) studied the effect of the organophosphorus insecticides fonofos and parathion on different soil-inhabiting microorganisms over seven days. They reported that populations of fungi, N₂-fixers, and denitrifiers were less than similar populations in untreated controls. However, nitrifiers (both ammonium and nitrate oxidizers) were not affected. The organophosphorus insecticide chlorpyrifos has been shown to inhibit growth of denitrifiers, aerobic N₂-fixers, and aerobic phosphorus dissolvers, but stimulate growth of nitrifiers, anaerobic N₂-fixers, phosphorus dissolvers, actinomycetes, ammonifiers, cellulose decomposers, sulfate reducers, and iron precipitators (Sivasithamparam, 1970). Wood and McRae (1974) noted that chlorpyrifos did not affect nitrogen-fixers (Azotobacter vinelandii), however, the major hydrolytic product of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCP), did have significant inhibitory effect on the nitrogen fixer population studied. Both Sivasithamparam (1970) and Wood and McRae (1974) suggested that as TCP accumulates in soil, the uncoupling of cellular phosphorylation reaches a toxic level.

It becomes evident that the effects of pesticides on soil microflora are indeed variable. The variability observed is probably due to soil type, microbial profile, and most importantly, the structure of the pesticide, which dictates target metabolic pathways and the intensity of the dose-response to the various genera of microorganisms.
A common organophosphorus insecticide effect on soil microflora involves an initial inhibition period followed by recovery. Abdel-Mallek (1994) showed that the organophosphorus insecticide profenfos has an immediate inhibitory effect on soil fungal populations, but after approximately six weeks, fungal populations became similar to controls. Abdel-Mallek (1994) notes that in prior studies, chlorpyrifos, concentration between between 18 and 36 µg/g inhibited soil fungal populations (Abdel-Kader, 1976). Tu (1992) examined the effects on soil microorganisms caused by several herbicides. These herbicides adversely affected bacterial and fungal populations during the first week compared to untreated controls, but afterwards, populations became similar to those in the control. In the same study, application of atrazine significantly increased or stimulated microbial activity.
CHAPTER 3  METHODOLOGY AND FACILITIES

3.1 Methodology

The research presented in this Dissertation has been divided into six different Sections. Summarized below under each Project designation is a description of the experimental design and methodology of the experiments performed:

Project I  Studies On Demulsification and Sorption of Several Pesticides onto Lignocellulosic Matrices using Fluorometric and Gas Chromatographic Techniques

It appears that an emulsifiable concentrate acts a barrier to efficient/effective isolation of pesticides from emulsions. Several means of demulsification were examined to improve pesticide sorption, which included the addition of different salts. A fluorescent material 1,5-bis (5-phenyloxazolyl)-benzene (POPOP) was used as tracer in blank pesticide emulsions to model the behavior of pesticides. POPOP is a water insoluble compound similar to many of the water insoluble pesticides formulated as emulsifiable concentrates. It was believed that analyzing for POPOP by highly sensitive fluorometric techniques was a quicker method than analyzing for a particular pesticide by chromatographic techniques.

A Turner Model 430 fluorometer was used to detect the concentration of POPOP left in solution after the emulsion containing the POPOP had been exposed to a lignocellulosic material and various treatments to achieve good demulsification. The use of POPOP to model pesticide behavior was verified by chromatographic analysis of pesticides under similar conditions.

Project II  Comparison of Different Lignocellulosic Materials, Granular Activated Carbon, Granular Rubber, and Their Ability to Isolate Chlorpyrifos from an Emulsifiable Concentrate (Dursban® 4E) by Sorbative Processes

The Virginia Tech Pesticide Waste Disposal System (Figure 1) is based on isolating the pesticide phase from the aqueous phase of pesticide wastewater by a
combination of batch and column sorption processes. The sorbents used are lignocellulosic materials, which are ideal for the pesticide waste disposal system because they have good sorbative properties are good matrices for composting.

Freundlich sorption isotherm experiments were performed to compare the sorbative abilities of peat moss, steam-explo red wood, granular rubber, and granular activated carbon (GAC) to isolate chlorpyrifos formulated as Dursban® 4E. Additionally, treatments of demulsification and filtration were performed to determine their impact on the sorption of chlorpyrifos to various materials.

The Freundlich sorption isotherms are usually constructed by subjecting replicates of the same amount of sorbent to varying concentrations of analyte (compound being tested). The slope and y-intercept of a log-log plot of the amount of analyte sorbed versus the amount left in solution are Freundlich sorption isotherm coefficients. The resulting Freundlich sorption isotherm coefficients of various sorbents indicate the sorbative ability of the matrices, and can be used to compare the sorbative abilities of different matrices to each other. However, it is important for comparative purposes that the isotherms are constructed in the same manner.

The Freundlich sorption isotherms were constructed by keeping the Dursban® 4E emulsion the same (approximately 4331 µg/g chlorpyrifos) and varying the amount of sorbent. The effects on the isotherms of adding Ca(OH)₂ for demulsification and by filtering the solution were also studied. After 24 hours, the chlorpyrifos in the aqueous phase was partitioned into hexane, and gas chromatographic techniques were used to determine the amount of chlorpyrifos in each sample.

Project III The Fate of Atrazine and Chlorpyrifos in Several Field-Scale Composting Systems Designed to Degrade Pesticide Waste

The second major part of the Virginia Tech Pesticide Waste Disposal System (Figure 1) involves subjecting the isolated pesticide phase on the lignocellulosic materials to a biologically active composting environment. Through a combination of chemical and biological degradation, bound residue formation, and volatility, the pesticide phase is detoxified.

A series of experiments were performed to optimize the biological activity of compost and to accelerate pesticide dissipation. Four basic experiments were designed involving a variety of field-scale compost designs. Two of the experiments involved 189 L
bioreactors (Rubbermaid®). The dissipation of atrazine and chlorpyrifos in a peat moss-based compost was studied. The effect of different designs of the 189 L bioreactors on improving biological activity indicated by compost temperature was also studied.

A third series of experiments involved the dissipation of atrazine, chlorpyrifos, and malathion in two different compost designs. One compost design involved 20 L plastic bags (Ringer® Inc.) with holes for aeration. The other compost design was a one meter diameter compost confined with galvanized steel mesh fencing. Replicates of compost (10 g dry) treated with approximately 1000 µg/g of each pesticide were placed into several cloth (Rayon®) 10 by 10 cm bags. These were placed in the middle of each type of compost for retrieval at various time (i.e. 4, 8, 12, 16, and/or 20 weeks after experimental initiation). The biological activity was monitored by recording compost temperatures electronically.

The contents of the cloth bags were extracted with ethyl acetate in 40 mL polypropylene centrifuge tubes. The amount of the pesticides in the extracts were determined by gas chromatographic or high performance liquid chromatographic techniques.

The final experiment involved monitoring atrazine, chlorpyrifos, and malathion dissipation in one cubic meter compost units over 36 weeks. The compost units were completely enclosed except for a screened bottom for air to enter the bulk of the compost, and two vents in the top for air to escape. Volatility of the pesticides was monitored for approximately one month to evaluate volatilization as a dissipation route. To trap volatile pesticides, polyurethane foam plugs were put into the exhaust vents and removed periodically for extraction with cyclohexane and pesticide analysis by gas chromatographic techniques.

Project IV The Effect of Bioturbation by Mite Activity on Atrazine Dissipation in Compost

It was interesting to observe large population of mites in several of the field-scale compost designs studied. It appears that mites are one of the major invertebrate inhabitants of compost (Polprasert, 1989), being found in the cooler outer regions. Studies were conducted in coordination with a class project in insect ecology to determine what effects that mite populations might have on increasing the dissipation of atrazine in compost. Also studied were the effects of compost hydration levels on mite populations,
and whether or not freezing compost material from the field killed mites. Having mites present in laboratory experiments have been a confounding factor and it was important to control them without chemicals.

Inocula of mites were collected from field compost and added to approximately 200 mL of a lignocellulosic material-based compost. Treatments included compost at 30, 50, and 70% hydration (water/wet matrix, w/w), and 5000 µg/g atrazine in a series of compost containers at 70% hydration. Replicate samples were set up to sacrifice samples for determining mite populations over time, as well as determining the concentration (µg/g) of atrazine over time. The samples were extracted in ethyl acetate in the 40 mL polyethylene centrifuge tubes, and the atrazine extracted and then quantified by gas chromatographic techniques.

**Project V  Effects of Nutrient Amendments on Atrazine and Chlorpyrifos Dissipation in an Aerobic Composting System Employing Lignocellulosic Materials Designed for Pesticide Disposal**

A laboratory experiment was performed to better determine the degree that nutrient amendments to compost have on increasing atrazine or chlorpyrifos dissipation. The biological activity was monitored by manometric measurements of oxygen consumption. The dissipation of atrazine and chlorpyrifos was monitored over a 16 week period. All of the samples were kept in an incubation chamber at approximately 50 °C to model optimal composting conditions (MacGregor et al., 1981).

Samples taken at the experimental initiation and at weeks 4, 8, and 16 were extracted with ethyl acetate in 40 mL polyurethane centrifuge tubes. Samples containing atrazine and chlorpyrifos were analyzed by high performance liquid chromatographic techniques. The major metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCP) and several of the atrazine metabolites were monitored to better determine the metabolism if the two pesticides in compost under optimal conditions of temperature, hydration, and biological activity. The atrazine metabolites monitored were hydroxyatrazine, de-ethyl-atrazine, desopropyl-atrazine, de-ethyl-hydroxyatrazine, desopropyl-hydroxyatrazine, and dealkylated atrazine.
Project VI  Fate of $^{14}$C-Atrazine and $^{14}$C-Chlorpyrifos under Laboratory Conditions in Compost Bioreactors Designed as a Pesticide Waste Disposal System

A series of experiments involving radiolabeled $^{14}$C-atrazine and $^{14}$C-chlorpyrifos under laboratory conditions were performed to determine the fate of each pesticide in compost. The experiments were performed using the laboratory apparatus designed by Petruska et al. (1985). This apparatus allowed efficient trapping of volatiles released from compost as carbon dioxide (mineralization) or from volatilization of the parent pesticide compound or metabolites. It is advantageous to use radiolabeled compounds, because the fate of the radiolabeled compounds can be accurately traced by radiometric techniques, which cannot be done with normal pesticide residue analyses. In theory, 100% of the radioactivity put into the system should be able to be recovered from the system, and the knowledge of what fractions the radioactivity is found can give important insights into the physicochemical behavior of the compound. The effects of adding corn meal and nitrogen, phosphorus, potassium (NPK) fertilizer to the compost on the fate of the $^{14}$C-atrazine and $^{14}$C-chlorpyrifos in compost were also examined.

Radiolabeled $^{14}$C-atrazine and $^{14}$C-chlorpyrifos were mixed with their respective formulations, AAtrex® 4L and Dursban® 4E, and applied to 10 g of a dry mixture in a specially designed glass bioreactor, resulting in approximately 5000 $\mu$g pesticide/g dry matrix and 4 $\mu$Ci/10 g dry matrix. To certain replicates, corn meal and NPK fertilizer were added to the bioreactors. This process was repeated every four weeks. Between 1 and 5 mL of tap water was added to the compost once a week to maintain the hydration at point where water began pool at the bottom of the test vessel. Air was pumped through the bioreactor through a series of polyurethane foam plugs to trap volatile organics and then through 5 M KOH traps for carbon dioxide. Samples were taken for extraction with organic solvents on the first day of the experiment and at weeks 4, 8, and 16 after experimental initiation. After the solvent extraction, the matrix was extracted with 0.1 M NaOH. The NaOH extract was acidified with sulfuric acid to a pH of 2, and the resulting precipitate represented the humic acid fraction and the aqueous portion represented the fulvic acid fraction. The radioactivity in the humic acid fraction and the leftover matrix was collected by combustion techniques and quantified by liquid scintillation counting. The radioactivity associated with each of the following fractions was determined and tabulated: (1) organic volatiles, (2) carbon dioxide, (3) organic solvent extract, (4) humic acid, (5) fulvic acid, and (6) the amount left associated with the matrix. Additionally, the
fate of the parent pesticides was monitored by high performance thin-layer chromatography and liquid scintillation counting (LSC) of the zonal scrapings.

3.2 Facilities and Equipment

All laboratory research was conducted at Virginia Polytechnic Institute and State University (Virginia Tech) in Blacksburg, Virginia. Laboratory work was done in several laboratories at Virginia Tech, which included the Pesticide Residue Laboratory of the Department of Biochemistry and Anaerobic Microbiology, the Department of Crop and Soil Environmental Sciences, the Department of Environmental Engineering, and the Department of Biological Systems Engineering. Field research was conducted at the Virginia Tech Turf Grass Research Center. Research involving a specially made chamber for maintaining high temperature (50 °C) was carried out in the Virginia Tech greenhouses.

The laboratory apparatus as described by Petruska et al. (1985) for collecting radicelabeled volatiles from glass containers was located in the Department of Entomology.

Most preparatory lab work and bench chemistry was completed in the Department of Entomology. Generally, pesticides in compost matrix were extracted with either acetone, ethyl acetate, or methanol:water (80:20, v:v). If a Polytron ultrasonicator was used to extract pesticides, the compost/pesticide combination was put into 500 mL French square bottles with the extraction solvent. If a sonicating water bath was used, approximately 1 g (dry weight) matrix was put into 40 mL polypropylene centrifuge tubes along with the extraction solvent. At least three separate extractions were done for each sample, and in the case of the 14C-radiolabeled experiments, exhaustive extractions were completed. Extracted samples were centrifuged (1687 RCF relative centrifugal force) between each extraction with a Beckman centrifuge located in the Department of Entomology. Supernatant from centrifuge tubes, as well as solvents from the French square bottles, were decanted into separate vessels. These decanted solvents were either concentrated under a nitrogen stream or allowed to concentrate in an exhaust hood. The samples at this point either were taken to near dryness and then reconstituted with hexane or ethyl acetate for analysis by gas chromatography (GC), or taken directly for analysis by high performance liquid chromatography (HPLC), or taken directly for analysis by high performance thin-layer chromatography (HPTLC).
Analysis of fluorescence was performed using a Turner 430 variable wavelength fluorometer located in the Department of Entomology. Samples were placed into quartz cuvettes for analysis and compared to solvent blanks.

A variety of chromatographic instruments were used during the research and are listed below. Specific analytical instrument parameters are detailed in the particular Project to which they apply. A Tracor 540 gas chromatograph with electron capture detector and Hewlett Packard high performance liquid chromatograph (HPLC) were located in the Department of Entomology. The Tracor 540 was used to analyze for atrazine, chlorpyrifos, diazinon, and malathion. A 1.8 m glass column (0.635 cm, internal diameter) was used with one of two packings. One column used was packed with Supelcoport® 100 - 120 mesh (1.5 - 1.95%, SP2250 - SP2401) to separate malathion, atrazine, and chlorpyrifos. The other column was packed with OV-17/OV-210 (1.5%/1.95%) liquid phase on a 100/120 mesh Chromosorb W HP stationary phase (Supelco).

The HPLC unit in the Department of Entomology was a Hewlett Packard HPLC (Model No. 1050; Hewlett Packard Co., Avondale, PA) with a low pressure solvent-mixing unit, a Rheodyne (Coati, CA) Model 7125 injection valve fitted with a stainless-steel 20 µL injection loop, and an ultraviolet-visible (UV-VIS) detector (Hewlet-Packard Model No. 1050). The chromatographic column used for all analyses was a Nucleosil® C-18 HS-bonded silica column (250 mm long by 4.6 mm internal diameter, 5 µm particle-size, 60 Å pore-size, 18% w/w carbon load, endcapped silanols; Alltech).

The HPLC unit used in the Department of Crop and Soil Environmental Sciences was an Isco (Lincoln, NE) pump (Model 2350) and UV-VIS detector (Model V4). The column used for separating chlorpyrifos was an Isco Perisorb C-18 column (250 mm long by 4.5 mm internal diameter, 5 µm particle size).

One of two different gas chromatographs (GC) were used to analyze for carbon dioxide, oxygen, and carbon dioxide. One GC was a GOW-MAC (Ottawa, Canada) fitted with two different 1.8 m long 0.318 cm internal diameter) copper columns. One column containing Haysep packing (Supelco, Inc., Belfonte, PA) was used for separating CO₂ and CH₄. The other column containing molecular sieve 5A (45-60 mesh, Sigma Chemical, Chicago, IL) was used for separating O₂. The carrier gas used was helium.

The other GC was a Hewlett Packard model 5890A equipped with two different 1.8 m long 0.318 cm internal diameter) stainless steel columns. One column was packed with Poropak N (80/100 mesh) for separating CO₂ and CH₄. The other column was
packed with molecular sieve 5A (45-60 mesh, Sigma Chemical) was used for separating \( \text{O}_2 \). Helium was used as the carrier gas.

High performance thin-layer chromatographic (HPTLC) was used in some circumstances. Normal phase silica gel type F or G HPTLC plates (Merck) were used to separate pesticides. In general, aliquots of an extract were placed into 1.5 mL polypropylene microcentrifuge tubes and taken to near dryness with a stream of nitrogen. Between 5 and 100 \( \mu \text{L} \) of ethyl acetate was added to each tube, the contents mixed, and then between 5 and 10 \( \mu \text{L} \) applied to the HPTLC plate by capillary pipette.
CHAPTER 4  RESULTS AND DISCUSSION

4.1 PROJECT I  Studies on Demulsification and Sorption of Several Pesticides onto Lignocellulosic Matrices using Fluorometric and Gas Chromatographic Techniques

4.1.1  Abstract

A pesticide waste disposal system using sorption/filtration and microbial degradation designed for pesticide applicators is being developed. The system employs lignocellulosic materials which remove pesticides from aqueous suspensions by sorption and filtration. The sorbed and filtered products are then placed in a bioreactor provided with nutrients, and the pesticide molecules are degraded by microbes.

The sorption of 5000 µg/g chlorpyrifos (Dursban® 4E; Dow-Elanco) and diazinon (Diazinon® 2E; Ciba-Geigy) was increased from approximately 40% to above 90% onto steam-exploded lignocellulosic wood products with the addition of Ca(OH)₂. However, studies concerned with optimizing demulsification and sorption of formulated pesticides proved to be time-consuming using conventional gas chromatographic techniques. In an attempt to expedite laboratory studies, the use of a fluorescent compound, 1,5-bis (5-phenyloxazolyl)-benzene or POPOP, as a pesticide model has been examined. Addition of POPOP to emulsifiable concentrates followed by dilution with water provided stable pesticide and POPOP emulsions which could be studied using fluorometric techniques. The disappearance of POPOP fluorescence from a demulsified solution correlated closely with pesticide sorption. The developed fluorometric technique and gas chromatographic techniques were used to study and optimize the demulsification of several pesticide emulsifiable concentrate formulations and their subsequent sorption onto various lignocellulosic wood products or activated carbon. Demulsification of various pesticide emulsifiable concentrates required the addition of salt (Na₂SO₄, CaCO₃, Ca(OH)₂). The addition of Ca(OH)₂ at 30 mg/mL of emulsion resulted in the best demulsification. Also, lignocellulosic wood products appeared to be comparable to activated carbon in their sorbative qualities.

Major advantages of using the fluorometric technique developed here to evaluate demulsification/sorption processes is that it allows for: (1) rapid evaluation of emulsion
stability; (2) sensitive measurements in the range of ng/L to mg/L, and, (3) evaluation of demulsification of emulsions which may contain a variety of unknown compounds and adjuvants.

4.1.2 Introduction

Health and environmental aspects of pesticides have been a concern to the public, political groups, and the scientific community since the advent of pesticide use. Major concerns regarding persistence and ecological impacts of certain pesticides during the 1970's resulted in the restriction of DDT and related chlorinated hydrocarbon pesticides. Recently, concerns have turned to groundwater quality and the detection of unacceptable concentrations of pesticides and chemicals produced from human activities. The potential pesticide threat to human health and the environment has resulted in efforts focused on the reduction and resolution of this problem.

Pesticides present in groundwater can originate from point or non-point sources. Non-point sources, such as normal farming applications, are widespread and probably constitute the majority of overall groundwater contamination by pesticides (Hallberg, 1989). However, point source groundwater contamination by pesticides is considerable and can constitute a higher concentration of pesticide reaching the groundwater supply. Larger amounts of pesticide can be released in a smaller area due to accidental or deliberate spills onto the ground, but the contamination is usually localized. Some important point sources include certain pesticide manufacturing sites, storage and transfer areas, formulation centers, transport, and mixing or disposal sites. In intensive agricultural areas, such as the Midwestern US corn belt, approximately 1500 different point sources have been identified (Hallberg et al., 1986; Long, 1988; and Hallberg, 1989). On the farm, major point sources consist of spills, back-siphoning and deliberate disposal (Hallberg, 1989).

Proper disposal of pesticide-laden water resulting from the clean up of pesticide application equipment or left over from a tank-mix to spray crops as an example, can be a problem for most pesticide applicators. Because of today's environmental concerns, applicators need a safe, inexpensive and effective means for the proper disposal of pesticides. Incineration, rinsate reuse, and burial of pesticide waste sorbed onto activated carbon are some of the major methods utilized to dispose of pesticide waste (Kreuger and
Several alternative methods for pesticide waste disposal are being developed that degrade the pesticide to harmless byproducts by physical, chemical and/or biological means. Examples of the alternative methods include the use of UV-light (Crosby and Wong, 1977; Plimmer, 1977), UV-light and ozonolysis (Kearney et al., 1984), hydrolysis (Kennedy et al., 1969; Dennis, 1972) or enzyme reduction (Honeycutt et al., 1984). Those alternative disposal methods available to pesticide applicators are effective, but their cost or dependence on rather complex technology restricts their widespread use.

A pesticide waste disposal system based on sorption and biodegradation of the pesticide is under development and is designed to provide an effective, inexpensive, and fairly easy process for those requiring disposal of small quantities of concentrated or large volumes of dilute pesticides (Hetzel et al., 1989; Mullins et al., 1989). Formulated pesticides contained in an aqueous phase are sorbed or isolated with the organic matrix during filtration. The organic matrix containing the isolated pesticides are then placed in a bioreactor where microorganisms degrade the pesticides to less complex molecules or non-toxic products.

Emulsifiable concentrates are the most predominant pesticide formulation used in U.S. Agriculture (Becher, 1985; Ware, 1994). Emulsifying agents allow for the suspension of hydrophobic pesticides in the aqueous phase. These emulsifying agents are essential in providing for effective pest control performance, but it has been shown in preliminary work that emulsions complicate wastewater cleanup procedures because they interfere with pesticide sorption onto organic matrices. To resolve this problem, it was found that demulsification techniques enhance the sorption process, and significantly improve pesticide removal from the aqueous phase.

To study and optimize techniques for pesticide demulsification and to improve pesticide sorption, a rapid and sensitive means of demulsification evaluation was proposed. The use of a fluorescent compound has been added to pesticide formulations in place of, or in addition to, pesticides in order to mimic the performance of hydrophobic pesticides during demulsification and subsequent sorption. It was thought, that in initial demulsification studies, fluorometric techniques could provide information about demulsification and sorption of hydrophobic molecules present in an emulsion more quickly and efficiently than if traditional chromatographic techniques for pesticides were utilized.
4.1.3 Materials and Methods

Laboratory protocol using the fluorescent probe was designed to resemble anticipated disposal practices. These include: 1:40 ratio of sorbent weight to the weight of wastewater to be treated; mechanical agitation for variable time intervals (4 to 24 hours); separation of the aqueous phase from the solid phase by filtration, and discarding the filtrate or treating the filtrate before discarding. The filtered residue (sorbent and pesticide) is then placed into bioreactors to allow for pesticide chemical degradation and/or biodegradation.

A fluorescent compound, 1,5-bis (5-phenyloxazolyl)-benzene or POPOP, having similar solubility characteristics as many hydrophobic pesticides, was selected to act as the representative pesticide probe. Aliquots of emulsifiable concentrate pesticide formulations (EC plus pesticide) or formulated blanks (EC minus pesticide) were placed along with an 18.6 µL aliquot of 275 µg/g POPOP (hexane) into a 25 mL scintillation vial. Malathion (O,O-dimethyl phophorodithioate) was formulated as Dragon® 4E (Mobay Corp.), diazinon (O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidy1) phosphorothioate) was formulated as Diazinon® 4E (Ciba-Geigy), and chlorpyrifos (O,O-dieethyl-O-(3,5,6-trichloro-2-pyridyl) phosphothioate) was formulated as Dursban® 4E (Dow-Elanco). An emulsifiable concentrate blank was supplied by the Mobay Corp. as a blank of Baygon® 1.5E (i.e. Baygon® EC without the pesticide propoxur).

Distilled water was then added resulting in a 10 mL volume with a pesticide concentration equivalent to 5000 µg/g, which is representative of recommended application rates of some EC pesticide formulations. Two hundred and fifty milligrams of sorbent (peat moss, steam-exploded wood, pine bark mulch or activated carbon) were then added to the vial, and the contents mixed (vortex) for several minutes, forming a stable emulsion. The peat moss was Canadian sphagnum peat moss. The steam-exploded wood (yellow poplar; *Liriodendron tulipifera* L.) was provided by the Virginia Tech Biobased Material Research Center (Blacksburg, VA) through a process described by Overend and Chornet (1987) involving a Masonite® process gun. The pine bark (mulch) was a combination of shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) bark. The activated carbon was Calgon brand Chromosorb® 200.

The demulsification treatment was done by adding various salts (Na₂SO₄, CaCO₃, Ca(OH)₂) in amounts ranging from 0.038 g to 1 g and then by mixing the slurry on a
shaker table for 4 to 6 hours. The solution was mixed vigorously using a vortex mixer and vacuum-filtered using a Buchner funnel with a Whatman #2 filter paper. The filtered sorbent with the filter was placed into 250 mL hexane and the pesticide extracted with a high speed grinder probe (Polytron®) at about 25% maximum power for 5 min. Three hundred microliter aliquots of the sorbent extract or filtrate were placed into 15 mL glass centrifuge tubes containing 1 g Na₂SO₄, mixed and diluted to 6 mL with hexane. The tubes were vortexed and centrifuged (1000 x g for 2 min), and 2 mL of the upper hydrophobic hexane phase were transferred to quartz glass cuvettes for fluorometric analysis. The fluorescence was recorded using a Turner 430 fluorometer (excitation wavelength= 357 nm; emission wavelength= 411 nm).

Two hundred fifty-microliter aliquots of the hexane/sorbent extract or the filtrate were diluted to 20 mL hexane for pesticide analyses. Additional sample preparation for pesticide analyses included addition of three 20 mL (1:20 hexane:acetone) volumes followed by the addition of 2 or 3 drops of Keeper's solution (2% paraffin oil in hexane). The mixture was dried under nitrogen and the concentrated solution was then diluted with hexane to an appropriate volume for injection into a Tracer 540 gas chromatograph with a 6 ft glass column (1/4 inch i.d.) packed with Supelcoport® 100-120 mesh (1.5 - 1.95%, SP2250 - SP2401) equipped with an electron capture detector. Instrument temperatures were: injection port, 235 °C; column, 190 °C; detector, 350 °C; and carrier gas flow rate, 40 mL/min N₂.

4.1.4 Results and Discussion

Preliminary experiments indicated that pesticide removal in emulsions of malathion (Dragon® 50%, 4E), diazinon (Diazinon® 4E) and chlorpyrifos (Dursban® 4E) ranged from 30 to 90%. In several of these experiments, the degree of pesticide removal was often variable, and therefore, the results were inconclusive. Many factors could have been responsible for the variable results, such as pesticide hydrolysis, specificity, capacity, sorption quality of sorbent, and emulsion structure and stability. Pesticide hydrolysis was considered to be a beneficial factor in the disposal process, but other factors which might affect sorption were of interest. Therefore, emulsion structure disruption or demulsification by physical and/or chemical means should increase pesticide or POPOP sorption by removing the surfactant/micelle barrier between the sorbent suspended in the
aqueous phase and the internal pesticide (POPOP) phase. Studying various means of demulsification and sorption improvement by traditional chromatographic techniques would have required approximately a week before results could be available for comparison. However, the need for a quicker method of obtaining information on sorption improvement led to the development of the fluorescence probe model.

The POPOP method was used to study the effect of several demulsification treatments on the stability of the emulsion and POPOP sorption onto peat moss. Figure 4 illustrates the %POPOP sorption onto peat moss obtained by: (1) mixing on a rotating wheel (6 rpm, tilted at 45°); (2) low-energy sonication with mechanical mixing; (3) high-energy sonication with a sonicating cell disruptor; and (4) salt addition using 1 g Na₂SO₄. Salt addition provided the best emulsion disruption and POPOP sorption onto peat moss (4% of the original fluorescence remained in solution). This is not surprising, since the use of salt to prevent or disrupt emulsions is a standard laboratory practice. The other treatments provided little demulsification, because between 40 and 60% of the original fluorescence remained in solution, indicating little POPOP sorption had occurred. Other demulsification treatments which were tried included freezing, heating, and bubbling air through the emulsion (Lissant, 1983), but none of these treatments provided significant demulsification.

Based on these results, additional demulsification experiments were conducted using the POPOP method and various salt additions. The effects of 1.0 g Na₂SO₄ and 0.038 g CaCO₃ addition (15% of the amount of sorbent added) were examined on POPOP sorption (removal) from a propoxur emulsion blank (Baygon® blank; no propoxur, Mobay Corp.), emulsion onto peat moss, steam-exploded wood, pine bark or activated carbon (Table 1).

Activated carbon provided the best sorbency of POPOP in the absence of salt addition (63.9%; Table 1). Peat moss and the steam-exploded wood had similar sorbencies (53.5 and 52.9%, respectively) and the pine bark had the least POPOP sorbency of 43.4%. It appeared that mixing time influenced the degree to which POPOP was sorbed to the organic matrix without demulsification. The results of the experiment presented in Table 1 illustrates better sorbency of POPOP than in a preliminary experiment where peat moss provided the best POPOP sorbency (42.2%) followed by activated carbon (32.4%), pine bark (29.3%), and steam-exploded wood (19.5%). The experiment only involved one to two minutes of mixing, whereas another experiment involved vortexing for 10 seconds and then allowing the slurry to mix on the rotating wheel for two
Figure 4. POPOP\textsuperscript{2} Loss from Propoxur Emulsion-Blank\textsuperscript{3} Solutions using Various Demulsification Treatments

\textsuperscript{1}\% POPOP Remaining = \% relative fluorescence compared to POPOP control at 100%
\textsuperscript{2}POPOP is 1,5-bis(5-phenylazolyl)-benzene
\textsuperscript{3}Baygon 1.5E formulation blank contained all adjuvants except the propoxur insecticide

Vertical bars are standard error of the mean (n = 5)
Table 1  Comparison of Various Sorbents and Salting Treatment to Remove POPOP\(^1\) from Solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative Response(^2)</th>
<th>Percent Removal(^3)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsion Control</td>
<td>3.33</td>
<td>-</td>
<td>3.69 ± 0.09</td>
</tr>
<tr>
<td>Emulsion Blank</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hexane w/o POPOP</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Organic Matrices with POPOP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peat Moss</td>
<td>1.54</td>
<td>53.5 ± 0.6</td>
<td>2.46 ± 0.01</td>
</tr>
<tr>
<td>Peat Moss + Na(_2)SO(_4)</td>
<td>0.003</td>
<td>99.9 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>Peat Moss + CaCO(_3)</td>
<td>1.68</td>
<td>50.3 ± 0.6</td>
<td>4.54 ± 0.07</td>
</tr>
<tr>
<td>Pine Bark</td>
<td>1.87</td>
<td>43.9 ± 0.9</td>
<td>2.97 ± 0.01</td>
</tr>
<tr>
<td>Pine Bark + Na(_2)SO(_4)</td>
<td>0.06</td>
<td>98.1 ± 1.1</td>
<td>-</td>
</tr>
<tr>
<td>Pine Bark + CaCO(_3)</td>
<td>1.62</td>
<td>51.5 ± 0.6</td>
<td>5.45 ± 0.12</td>
</tr>
<tr>
<td>Steam-Exploded Wood (SEW)</td>
<td>1.57</td>
<td>52.9 ± 3.3</td>
<td>4.96 ± 0.04</td>
</tr>
<tr>
<td>SEW + Na(_2)SO(_4)</td>
<td>0.001</td>
<td>100.0 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>SEW + CaCO(_3)</td>
<td>1.79</td>
<td>47.6 ± 1.0</td>
<td>6.21 ± 0.03</td>
</tr>
<tr>
<td>Activated Carbon</td>
<td>1.2</td>
<td>63.9 ± 2.4</td>
<td>5.43 ± 0.03</td>
</tr>
<tr>
<td>AC + Na(_2)SO(_4)</td>
<td>0.18</td>
<td>94.6 ± 1.6</td>
<td>-</td>
</tr>
<tr>
<td>AC + CaCO(_3)</td>
<td>0.93</td>
<td>72.0 ± 1.6</td>
<td>7.66 ± 0.08</td>
</tr>
</tbody>
</table>

\(^1\)POPOP is 1,5-bis(5-phenyloxazolyl)-benzene

\(^2\)Results = Values are expressed as the mean ± standard error of the mean; n = 3

\(^3\)Treatment = after mixing by vortex mixer, 1 g Na\(_2\)SO\(_4\) or 0.031 g CaCO\(_3\) added, and both treatments mixed for 2 hrs

\(^4\)Relative response of fluorometer

\(^5\)Percent Removal = Percent decrease in fluorescence compared to fluorescence in Emulsion Control
hours. Increasing the amount of mixing time above two hours did not appear to increase POPOP sorption, while in another study, POPOP sorption did not significantly increase between two and 24 hours, mixing continuously on the rotating wheel (Student's t-test; \( \alpha = 0.05; n = 6 \)).

Salt addition (\( \text{Na}_2\text{SO}_4 \) or \( \text{CaCO}_3 \)) to the emulsion increased subsequent POPOP sorption. The emulsions were visibly disrupted with \( \text{Na}_2\text{SO}_4 \), but not so with \( \text{CaCO}_3 \). The \( \text{Na}_2\text{SO}_4 \) demulsification improved POPOP sorption to 99.1, 98.1, 100, and 94.6% for peat moss, pine bark, steam-exploded wood, and activated carbon, respectively. The apparent POPOP sorption improved slightly with \( \text{CaCO}_3 \) addition compared to those values associated without \( \text{Na}_2\text{SO}_4 \), however, those emulsions were not disrupted as with those treated by addition of \( \text{Na}_2\text{SO}_4 \). Differences in demulsification between these two salts was most likely due to their corresponding ionic strengths. Water solubility of \( \text{CaCO}_3 \) is 0.007 g/L (\( K_{sp} = 4.4 \times 10^{-9} \) for \( \text{CaCO}_3 \); Snoeyink and Jenkins 1980), whereas the water solubility of \( \text{Na}_2\text{SO}_4 \) is approximately 440 g/L (CRC 1978). Since greater demulsification is achieved by increased ionic strengths (Becher, 1985), \( \text{Na}_2\text{SO}_4 \) in this system, which had an ionic strength of 8.4 should have provided better demulsification than \( \text{CaCO}_3 \), which had an ionic strength of 0.0007.

It was found that the POPOP fluorescence analyses provided considerable time savings compared to routine pesticide analysis by conventional chromatographic techniques. As a result, POPOP behavior as a pesticide probe was examined along with different treatments for improving demulsification and sorption. The question of the correlation between POPOP removal and hydrophobic pesticide removal during demulsification was addressed. The data in Table 2 present information on the separate quantitation of POPOP (fluorometric analysis) and diazinon (GC analysis) in solution from the same treatments. Either peat moss or steam-exploded wood was used as a sorbent and demulsification was achieved using 1.0 g \( \text{Na}_2\text{SO}_4 \).

POPOP and diazinon sorbencies appeared to be similar when treated in a similar manner (Table 2). POPOP and diazinon sorption values, both with and without the addition of \( \text{Na}_2\text{SO}_4 \), were similar except in the peat moss control where the POPOP removal/sorption value (49%) was higher than the diazinon sorption value (35%). Reasons for this difference could be due to the pH-related peat moss destabilization of the emulsion allowing for differences in POPOP or diazinon removal/sorption onto the peat moss. Without the destabilization effect of salt addition, the emulsion destabilization
Table 2  Comparison of POPOP\(^1\) and Diazinon Removal from Solution by Lignocellulosic Sorbents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sorbate</th>
<th>Percent Removal from Solution(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peat Moss</td>
</tr>
<tr>
<td>POPOP(^3)</td>
<td>49% ± 0.8%</td>
<td>48% ± 1.1%</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazinon(^4)</td>
<td>35% ± 0.5%</td>
<td>46% ± 1.2%</td>
</tr>
<tr>
<td>Na(_2)SO(_4) Addition(^5)</td>
<td>88% ± 0.6%</td>
<td>88% ± 0.5%</td>
</tr>
<tr>
<td>Diazinon</td>
<td>87% ± 0.6%</td>
<td>91% ± 1%</td>
</tr>
</tbody>
</table>

\(^1\)POPOP is 1,5-bis(5-phenyloxazolyl)-benzene
\(^2\)Values are expressed as the mean ± standard error of the mean; n = 5
\(^3\)POPOP, 5.1 µg/10 mL
\(^4\)5000 mg/kg diazinon as Diazinor® 4E (Ciba Geigy)
\(^5\)1.0 g Na\(_2\)SO\(_4\) was added as the demulsification agent; the solution was mixed for 2 hrs prior to analysis
appears to be related to the extent that the micelles interact with different sorbents (Ross and Morrison, 1988). If the sorbent is strongly attractive to the surfactants, micelles could be completely disrupted. It does not appear that peat moss, pine bark, steam-exploded wood, or activated carbon disrupted the emulsion integrity well enough to allow for the internal POPOP molecules to be sorbed. The possibility that POPOP might interfere with pesticide (diazinon) removal/sorption during demulsification was also examined. Incorporation of POPOP into a diazinon emulsion does not appear to significantly change diazinon sorption in the presence of POPOP (Table 3). The removal of diazinon from Diazinon® 4E emulsions with and without POPOP present was similar in both of the sorbents examined: peat moss (87 ± 0.6%) or steam-exploded wood (91 ± 1%).

Up to this point, it was assumed that POPOP/diazinon removal could be closely correlated with sorption. To examine this assumption more closely, a series of experiments were completed to estimate POPOP/diazinon recoveries in the filtrate and on the sorbent after the demulsification step. After demulsification and filtration, the total percent recoveries were 14.2 ± 3.5%. This low recovery required explanation, and further experimentation indicated that the POPOP was not chemically altered (hydrolyzed) in similar solutions without the sorbent. It was assumed that a significant amount was bound to the steam-exploded wood, and approximately 75% of that phase was unextractable. The concentration of POPOP (5.12 µg/g) and 300 mg Ca(OH)₂ did not change significantly over 24 hours after the Ca(OH)₂ had been added, indicating hydrolysis did not affect the concentration of POPOP. A control containing Diazinon® 4E (emulsion) and POPOP without a sorbent was demulsified with 300 mg of Ca(OH)₂. After rinsing and extracting the glassware and demulsified solution, 88 ± 7% of the POPOP was recovered. It was possible that there was something extracted from the lignocellulosic matrices that quenched the POPOP fluorescence. However, experiments were not performed to determine possible quench effects from the matrices. It is also possible that POPOP binds with the matrices in such a way that it is not recovered in the hexane extraction. Whatever the case may be for the poor POPOP recoveries, the POPOP fluorescence method described here did appear to model diazinon sorption well as evident in Table 2.

Similar results were obtained in studies using chlorpyrifos (Dursban® 4E) and steam-exploded wood and provided total recovery values ranging between 50 and 70%.
Table 3  Removal\(^1\) of Diazinon\(^2\)-4E with and without the Addition of POPOP\(^3\) after Demulsification\(^4\)

<table>
<thead>
<tr>
<th></th>
<th>Peat Moss</th>
<th>Steam-exploded wood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>w/POPOP</td>
</tr>
<tr>
<td></td>
<td>87% ± 0.6%</td>
<td>87% ± 0.5%</td>
</tr>
</tbody>
</table>

\(^1\)Percent of removal/sorption expressed as the mean + standard error of the mean; \(n = 3\)

\(^2\)Diazinon as Diazinon\(^6\)E at an initial concentration of 5000 mg/kg

\(^3\)POPOP - 1,5-bis(5-phenyloxazolyl)-benzene

\(^4\)1.0 g Na\(_2\)SO\(_4\) was added as the demulsification agent; the solution was mixed for 2 hrs prior to analysis
These values were increased to nearly 100% when the solution was demulsified Ca(OH)$_2$ in the absence of the sorbent indicating that there was an unextractable portion when the sorbent was present.

Since the POPOP probe was found to mimic hydrophobic pesticides during demulsification and sorption processes, a larger and more comprehensive study was completed using various salts (Na$_2$SO$_4$, Ca(OH)$_2$, CaCO$_3$) and sorbents (peat moss and steam-exploded wood). Table 4 presents the data on POPOP sorption onto peat moss or steam-exploded wood with the POPOP probe incorporated in 5000 µg/g chlorpyrifos (Dursban® 4E), malathion (Dragon® 4E), or diazinon (Diazinon® 4E) formulated emulsion. In most cases, demulsification appeared to be complete (visual approximation) and the subsequent POPOP removal/sorption was 85% or higher, irrespective of pesticide type or salt addition. However, low POPOP sorption occurred and correlated well in a few solutions that did not visibly appear to have complete demulsification. The addition of 300 mg CaCO$_3$ to all insecticide emulsions with peat moss, 1 g Na$_2$SO$_4$ to chlorpyrifos with peat moss, and 100 mg Ca(OH)$_2$ to diazinon with peat moss did not appear to provide complete emulsion disruption, and the POPOP sorption values were 75% or below. However, only the diazinon emulsion mixed with steam-exploded wood and 300 mg CaCO$_3$ did not completely demulsify, resulting in 69% POPOP removal/sorption. The other two insecticide emulsions (chlorpyrifos and malathion) with steam-exploded wood and CaCO$_3$ did demulsify, and demonstrated high POPOP removal/sorption.

Reasons for poor demulsification and subsequent low POPOP sorption with CaCO$_3$ may have been due to a combination of low CaCO$_3$ solubility (0.007 g/L; Snoeyink and Jenkins, 1980), and the influence of the initial pH. The initial pH in a slurry of 250 mg peat moss in 10 mL water was approximately 3, and the addition of 300 mg/mL CaCO$_3$ to insecticide emulsions mixed with peat moss did not increase the pH above 5.8. However, the pH did rise to approximately 7 when CaCO$_3$ and steam-exploded wood were mixed with the insecticide emulsions. Apparently, the initial pH and/or quality of sorbent might influence CaCO$_3$ solubility because the same emulsions (chlorpyrifos and malathion) were demulsified with steam-exploded wood where the initial pH values were approximately 5.5.

The addition of either 100 or 300 mg Ca(OH)$_2$ in the peat moss or steam-exploded wood treatments appeared to completely demulsify the insecticide emulsions in all but one case which involved diazinon, peat moss, and 100 mg of Ca(OH)$_2$ (Table 4). Ca(OH)$_2$ is more water soluble than CaCO$_3$ (1.27 g/L, Snoeyink and Jenkins, 1980) which may
### Table 4
Demulsification and POPOP Sorption onto Pest Moss and Steam-Exploded Wood in Various Pesticide Emulsifiable Concentrate Formulations Treated with Different Salts

#### Pest Moss

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%POPOP Removed from Solution&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Emulsion State after Adding Salt&lt;sup&gt;4&lt;/sup&gt;</th>
<th>pH Before / After&lt;sup&gt;7&lt;/sup&gt;</th>
<th>%POPOP Removed from Solution&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Emulsion State after Adding Salt&lt;sup&gt;4&lt;/sup&gt;</th>
<th>pH Before / After&lt;sup&gt;7&lt;/sup&gt;</th>
<th>%POPOP Removed from Solution&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Emulsion State after Adding Salt&lt;sup&gt;4&lt;/sup&gt;</th>
<th>pH Before / After&lt;sup&gt;7&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; 1g</td>
<td>77.6 ± 8.5</td>
<td>ND</td>
<td>3.02 / 3.50</td>
<td>89.7 ± 0.3</td>
<td>D</td>
<td>3.05 / 4.43</td>
<td>84.9 ± 0.7</td>
<td>D</td>
<td>3.03 / 3.42</td>
</tr>
<tr>
<td>Ca(OH)&lt;sub&gt;2&lt;/sub&gt; 100 mg</td>
<td>89.8</td>
<td>D</td>
<td>3.01 / 11.67</td>
<td>93.6 ± 1.0</td>
<td>D</td>
<td>3.04 / 11.23</td>
<td>72.8 ± 9.0</td>
<td>ND</td>
<td>3.04 / 11.49</td>
</tr>
<tr>
<td>Ca(OH)&lt;sub&gt;2&lt;/sub&gt; 300 mg</td>
<td>98.2 ± 0.3</td>
<td>D</td>
<td>3.09 / 11.60</td>
<td>99.1</td>
<td>D</td>
<td>3.05 / 11.62</td>
<td>99.1 ± 0.05</td>
<td>D</td>
<td>3.02 / 11.84</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt; 300 mg</td>
<td>69</td>
<td>ND</td>
<td>3.04 / 5.85</td>
<td>75.0 ± 4.2</td>
<td>ND</td>
<td>3.04 / 5.80</td>
<td>65.5 ± 1.4</td>
<td>ND</td>
<td>3.04 / 5.67</td>
</tr>
</tbody>
</table>

#### Steam-exploded wood

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%POPOP Removed from Solution&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Emulsion State after Adding Salt&lt;sup&gt;4&lt;/sup&gt;</th>
<th>pH Before / After&lt;sup&gt;7&lt;/sup&gt;</th>
<th>%POPOP Removed from Solution&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Emulsion State after Adding Salt&lt;sup&gt;4&lt;/sup&gt;</th>
<th>pH Before / After&lt;sup&gt;7&lt;/sup&gt;</th>
<th>%POPOP Removed from Solution&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Emulsion State after Adding Salt&lt;sup&gt;4&lt;/sup&gt;</th>
<th>pH Before / After&lt;sup&gt;7&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; 1g</td>
<td>97.7 ± 0.04</td>
<td>D</td>
<td>5.28 / 6.13</td>
<td>98.8 ± 0.6</td>
<td>D</td>
<td>5.33 / 6.19</td>
<td>96.3 ± 0.4</td>
<td>D</td>
<td>5.42 / 6.23</td>
</tr>
<tr>
<td>Ca(OH)&lt;sub&gt;2&lt;/sub&gt; 100 mg</td>
<td>98.5 ± 0.8</td>
<td>D</td>
<td>5.17 / 11.25</td>
<td>99.3 ± 0.3</td>
<td>D</td>
<td>5.28 / 10.94</td>
<td>89.5 ± 0.4</td>
<td>D</td>
<td>5.47 / 11.21</td>
</tr>
<tr>
<td>Ca(OH)&lt;sub&gt;2&lt;/sub&gt; 300 mg</td>
<td>99.1 ± 0.3</td>
<td>D</td>
<td>5.32 / 11.45</td>
<td>98.8 ± 0.4</td>
<td>D</td>
<td>5.44 / 11.24</td>
<td>95.5 ± 0.5</td>
<td>D</td>
<td>5.55 / 11.37</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt; 300 mg</td>
<td>94.4 ± 1.0</td>
<td>D</td>
<td>5.37 / 7.17</td>
<td>96.9 ± 0.05</td>
<td>D</td>
<td>5.60 / 7.03</td>
<td>69.0 ± 0.4</td>
<td>ND</td>
<td>5.66 / 6.85</td>
</tr>
</tbody>
</table>

1. Chlorpyrifos formulated as Durban<sup>4</sup>E
2. Malathion formulated as Dragon<sup>4</sup>E
3. Diazinon formulated as Diazinon<sup>4</sup>E
4. Treatment addition of salt to disrupt emulsion
5. % POPOP loss represents POPOP sorption onto sorbent ± standard error of the mean (n = 3 or 4)
6. State of emulsion after Adding Salt (D = Emulsion visibly disrupted; ND = Emulsion not visibly disrupted)
7. pH before & after salt addition
explain the increase in demulsification with smaller amounts of Ca(OH)$_2$ compared to CaCO$_3$. There appeared to be an increase of POPOP sorption with an increase of 100 to 300 mg Ca(OH)$_2$. This may have been a result of the divalent Ca$^{2+}$ cation being present. Divalent cations have been shown to combine with organic matter and/or pesticides (Stevenson 1982). In addition to normal sorption processes, the complexing processes may have been involved in removing additional POPOP associated with soluble organic matter or particulate organic matter, increasing the apparent amount sorbed onto peat moss or steam-exploded wood.

The addition of 1 g Na$_2$SO$_4$ to the peat moss and steam-exploded wood mixtures appeared to disrupt all but the chlorpyrifos emulsion with peat moss (Table 4). All of the Na$_2$SO$_4$ (1 g) should have been dissolved in 10 mL, but, in general, the sorption was not as good as with lower amounts of Ca(OH)$_2$. The monovalent Na$^+$ ions may be adequate demulsifying agents, but may not act as well as divalent cations in complexing pesticides and/or organic matter.

Based on our results using salts as pesticide demulsifying agents, Ca(OH)$_2$ was selected for further study. The Ca(OH)$_2$ (10 - 30 g/L) appeared to effectively demulsify those insecticide emulsions studied as presented in Table 4. It should also be noted that Ca(OH)$_2$ appears to be a sensible candidate as a demulsifying agent for use in field disposal applications. Ca(OH)$_2$ or burnt lime is readily found in hardware stores or feed & seed stores at an affordable cost (approximately $3/25$ lb in 1989).

To examine the efficiency of pesticide removal using various sorbents and Ca(OH)$_2$ as a demulsifying agent, experiments were performed on three pesticide formulations. It was believed that analyzing for pesticides during the demulsification/sorption processes would be better than assuming that POPOP would simulate the actual sorption characteristics of different pesticides in their respective formulations. Atrazine (AAtrex® 4L), chlorpyrifos (Dursban® 4E), and diazinon (Diazinon® 4E) at approximately 5000 µg/g were treated with 0.25 g of activated carbon, steam-exploded wood, pine bark, or peat moss in 100 mL water. The solutions were demulsified with 1 g Ca(OH)$_2$ and allowed to mix for 24 hours. The mixture was allowed to settle for 24 hours and was filtered using Whatman # 2 and #3).

In most cases, filtration reduced the amount of pesticide retained in solution (Table 5). The use of steam-exploded wood appeared to provide sorption values similar to those obtained with activated carbon. Atrazine sorption before filtration with activated carbon did not appear to occur, but did with steam-exploded wood (32.4%). As evident
Table 5  Comparison of Pesticide Removal from Various Pesticide Formulations Using Different Lignocellulosic Sorbents After Demulsification with Ca(OH)$_2$

<table>
<thead>
<tr>
<th>Pesticide$^2$</th>
<th>Activated Carbon</th>
<th>Steam-Exploded Wood</th>
<th>Peat Moss</th>
<th>Pine Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine$^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Filtered</td>
<td>87%</td>
<td>77.6% ± 11.8%</td>
<td>72.5% ± 10.3%</td>
<td>66.1% ± 24.3%</td>
</tr>
<tr>
<td>Filtered$^3$</td>
<td>0.6% ± 0.2%</td>
<td>1.1% ± 0.4%</td>
<td>1.3% ± 0.4%</td>
<td>1.3% ± 0.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chlorpyrifos$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Filtered</td>
</tr>
<tr>
<td>Filtered</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diazinon$^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Filtered</td>
</tr>
<tr>
<td>Filtered</td>
</tr>
</tbody>
</table>

$^1$Values are expressed as the mean ± standard error of the mean; percentage remaining based on detection limits in ppm, initial concentration was approximately 5000 ppm (mg/kg) for all pesticides

$^2$1 g Ca(OH)$_2$ added / 10 mL solution

$^3$AATREX$^R$ 4L, Ciba

$^4$Dursban$^R$ 4E, Dow-Detalco

$^5$Diazinon$^R$ 4E, Ciba
in Table 5, the action of sorption was improved by filtration, but sorption values were quite variable without filtering which may suggest that there is a spectrum of chemical and physical interactions between pesticide, sorption and filtration of pesticide particulates. Atrazine formulated as AAtrix® 4L is a particulate suspension which may help explain why atrazine sorption was poor (Table 5). Only the water soluble portion of atrazine would have been sorbed, whereas the particulate phase would have been removed by filtration. Diazinon and chlorpyrifos are formulated as emulsions and demulsification/sorption should account for the majority of pesticide removal, whereas filtration would remove that portion complexed to soluble organic matter and/or particulate organic matter.

The reason for the decreased presence of pesticide in solution after filtering could have been due to sorption of the pesticide onto the filter or containment of the pesticide-bound particulate portion of the slurry. Recoveries of only chlorpyrifos formulated as Dursban® 4E passed through a filter were 80 to 95%. If the filter was wet with water before filtration and rinsed afterward the recoveries improved to near 95%. The improvement probably occurred because the surfactant phase remained in the filter, but could be physically washed through the filter with water bringing associated pesticides with it. On the other hand, filtering chlorpyrifos which had been demulsified and allowed to sorb to steam-exploded wood resulted in over 30% of the original amount being associated with the filter, and this value was obtained after the filter was rinsed with water. Chlorpyrifos bound to the particulate matter and soluble organic matter (SOM) were thought to constitute a considerable component of the sorption phase. Both pesticides and POP appeared to bind to those phases in some fashion because when the demulsified pesticides were filtered, the majority of pesticide added had disappeared.

Filtering removed the largest percentage of particulate matter with all sorbents studied (Table 5), but did not necessarily remove the pesticide-bound SOM present in the organic sorbents. This appears to have been the case since those pesticides mixed with activated carbon appeared to decrease to near zero µg/g after filtration. A reason why activated carbon appeared to be a better sorbent compared to the organic sorbents could be explained by the SOM phase. Activated carbon does not contain a significant SOM phase and the pesticide-bound particulate phase appeared to have been removed during filtration (Table 5). Pesticide-bound SOM may also explain why diazinon sorption in the peat moss and pine bark treatments did not improve upon filtration. However, it could
have involved physical and chemical differences of the diazinon emulsion compared to the other emulsion studied.

4.1.5 Conclusions

In conclusion, the POPOP method had allowed the examination of several methods to improve sorption by demulsification processes and sorbent quality. Using the POPOP probe method, the results presented here indicate that demulsification could be an important step in improving POPOP sorption. The results using POPOP as an insecticide probe were similar to what occurred with regard to hydrophobic pesticide sorption. Ca(OH)$_2$ appeared to be the best demulsification agent due to its good solubility, the divalent Ca$^{2+}$ ion, high pH, and the availability and affordable cost to the potential non-industrial user of the pesticide disposal process which is being developed.

The importance of using a filter to remove the particulate phase was emphasized during the fluorometric and gas chromatographic techniques for POPOP or pesticide analyses. Because the sorbents used in this study were lignocellulosic fibers, there was a considerable amount of fine organic particulate matter and SOM present in the slurry. Further removal of this phase or the soluble portion of the pesticide may be required before these treated pesticide rinsates can be considered safe for release in the environment. In the cases where filtration was used, steam-exploded wood appeared to have comparable sorption qualities as activated carbon. If the pesticides associated with the SOM from steam-exploded wood were removed, then there was a possibility that the amount of pesticides in water would have been even lower. In any case, the combined procedures of demulsification and filtration appeared to remove the majority of pesticides studied by sorption and physical containment. The contained pesticide-organic sorbent phase then could be allowed to compost, chemically and biologically degrading the pesticides to non-toxic by-products and metabolites (Armstrong and Chesters 1968; Kearney and Karns 1987, Mullins et al., 1989)

The POPOP probe method allowed for a much shorter sample processing time when compared to standard gas chromatographic techniques. However, gas chromatographic techniques were required to determine the respective pesticide fates in specific formulations during demulsification and sorption. Although variable pesticide sorption values were obtained, depending on the sorbent used, the development and use of
the POPOP probe method enabled making more rapid progress in developing the demulsification procedure. It is quite possible that in the future, POPOP or other fluorescent probes could be used in the field to distinguish whether or not an emulsion has been disrupted. A quick check for disappearance of fluorescence after the demulsification treatment could help ensure optimal sorption conditions.
4.2 PROJECT II  Comparison of Different Lignocellulosic Materials, Granular Rubber, Granular Activated Carbon, and Their Ability to Isolate Chlorpyrifos from an Emulsifiable Concentrate (Dursban® 4E) by Sorption Processes

4.2.1 Abstract

Batch sorption studies were performed to compare the sorption capabilities of lignocellulosic materials, such as peat moss (PM) and steam-exploded wood (SEW), to granular activated carbon (AC) and granular rubber (R) for removing chlorpyrifos from an emulsion formulated as Dursban® 4E. The batch sorption process is the first of two processes which are involved in a pesticide waste disposal method being developed as an interdisciplinary project at Virginia Tech. The second process involves solid state fermentation (SSF) to detoxify the pesticide portion isolated from the batch sorption process through a combination of abiotic reactions and biotic metabolism.

An initial Dursban® 4E solution of approximately 4332 ± 323 μg/mL chlorpyrifos (± standard error of the mean, n = 8) was prepared and 10 mL portions added to triplicate 20 mL glass vials containing 0.025, 0.1, 0.25, 0.4, 0.5, and 1 g amounts of sorbent and also to control vials without sorbents. Treatments were demulsification by adding 300 mg Ca(OH)₂ followed by filtering (DF), demulsification with no filtering (DNF), no demulsification but filtration (NDF), or neither demulsification or filtering (NN). An additional treatment involved leaving vials of the initial Dursban® 4E solution undisturbed to examine the emulsion stability.

The appearance of the initial Dursban® 4E solution was cloudy and white. The emulsion of the initial Dursban® 4E solution was not stable over time. The appearance of the solution became less cloudy and precipitated material settled to the bottom of the vial by simply allowing it to settle for 18 hours. The concentration of chlorpyrifos in solution after the settling period was 1391 μg/mL, which translated to a 67% reduction of the original amount of chlorpyrifos in solution. The portion of chlorpyrifos not in solution was associated with the precipitated emulsion material. The remaining emulsion after the settling period was stable and approximated the hydrophilic lipophilic balance (HLB) of Dursban® 4E.

The addition of Ca(OH)₂ to the initial Dursban® 4E solution disrupted the
emulsion to a large extent as evident by the solution becoming less cloudy. As a consequence, the amount of chlorpyrifos in this unfiltered solution was greatly reduced to 9% (390 µg/mL) or a 91% removal of chlorpyrifos. The emulsion was not completely demulsified as indicated by the cloudiness of the solution and the concentration of chlorpyrifos (390 µg/mL) being above its water solubility of approximately 2 µg/mL. A completely demulsified solution would be clear.

The concentration of chlorpyrifos in solution in samples containing sorbents for all treatments ranged from 1330 ± 13 µg/mL (or 69.3% sorption; SEW; NDF at 0.025 g) to 2.1 ± 1.2 µg/mL (or 99.5% sorption; AC and R; DF at 1.00 g). The chlorpyrifos concentration of 1330 µg/mL with 0.025 g of SEW present did not differ much from those Dursban® 4E solutions allowed to remain undisturbed (1391 µg/mL). It appeared that there was a lower limit of sorbent present that could remove chlorpyrifos from the initial Dursban® 4E solution by sorption processes. Adsorption isotherm plots indicated unfavorable adsorption behavior for all sorbents and treatments. The batch sorption data did not fit the Freundlich adsorption isotherm model well as there were poor correlation coefficients and significant lack of fit for all isotherms to the Freundlich adsorption isotherm model. Using the Langmuir and Brunauer, Emmett and Tyler (BET) isotherm models did not improve the fit. The non-linear behavior of the isotherm was attributed to interference with the sorption of chlorpyrifos from the presence of an emulsion and surfactants in the Dursban® 4E solution.

The disruption of the Dursban® 4E emulsion (demulsification) proved to be an important step for decreasing the amount of chlorpyrifos in solution. The concentration of chlorpyrifos in solution decreased proportionally with the degree of demulsification indicated as an improvement in the solution clarity. Adding Ca(OH)₂ was an effective agent to promote demulsification. The aqueous concentration of chlorpyrifos ranged from 549 ± 88 µg/mL (or 87.3% Sorbed; SEW; DNF) to 2.2 ± 1.2 µg/mL (or 99.95% sorbed; AC; DF). Sorbents without Ca(OH)₂ present were also capable of disrupting the emulsion. All sorbents at the 1.0 g amounts without Ca(OH)₂ present resulted in clear solutions, but only R without Ca(OH)₂ present clarified the Dursban® 4E solutions at sorbent amounts less than 1.0 g (0.4 g lower limit).

Filtering the samples was more effective in reducing the aqueous concentration of chlorpyrifos in samples with the lignocellulosic sorbents than in samples with AC or R sorbents. Filtering removed the lignocellulosic particulates in solution and the portion of chlorpyrifos associated with the particulate phase. Filtering was not necessary with the
AC and R sorbents, since the sorbent and any associated chlorpyrifos settled due to the size and weight of the sorbent.

The DF treatment was the most effective treatment for all sorbents. In solutions that had Ca(OH)$_2$ present and were filtered (DF), the sorbents AC, R, and SEW were significantly similar in their ability to decrease the aqueous concentration of chlorpyrifos (P > 0.05). The aqueous concentration of chlorpyrifos for AC, R, and SEW ranged from $2.2 \pm 1.2 \mu g/mL$ (or 99.95%; AC at 1.0 g) to $259 \pm 25 \mu g/mL$ (or 94% sorption; SEW at 0.1 g). The concentration of chlorpyrifos was similar in samples containing lignocellulosic sorbents which had been only filtered to samples containing AC, R, and SEW after a DF treatment. As an example, there was an approximately 95% decrease in the aqueous concentration of chlorpyrifos in SEW and PM samples after a NDF treatment at sorbent amounts of 0.4 g or greater.

A desired concentration of chlorpyrifos in water would be below the maximum contaminant level (MCL) designated by regulatory units worldwide for chlorpyrifos in drinking water. Australia and Canada have set the MCL concentrations to be 0.002 and 0.09 $\mu g/mL$, which is lower than the results described here. It may be necessary to further treat the wastewater to remove chlorpyrifos to an acceptable concentration.

Lignocellulosic sorbents should be used as the primary sorbent in a batch sorption process involving pesticides that are similar to chlorpyrifos formulated as an emulsifiable concentrate. The bulk of the chlorpyrifos or similar product at typical pesticide-laden waste water concentrations greater than 4000 $\mu g/mL$ could be removed by PM or SEW in a batch sorption step followed by a secondary step of passing the wastewater through columns of AC or R. Refinement of the secondary sorption phase by using specific and specially designed sorbents may be necessary to effectively sorb all of the pesticide so that the water can be used in a non-potable way. It would be cost effective to use lignocellulosic materials for removing the bulk of pesticide from wastewater. Lignocellulosic materials are cost effective sorbents, readily available and are a renewable resource. Overloading specialized sorbent with the bulk of the waste pesticide may require frequent regeneration of the sorbent, which would be cost prohibitive. Additionally, the use of lignocellulosic materials to remove the bulk of the pesticide from pesticide-laden wastewater is appropriate in the pesticide waste disposal method being developed because they are part of the SSF process. The contaminated AC and R from secondary sorption processes can be added with the lignocellulosic materials to the SSF portion of the disposal process. Cleaning and detoxifying pesticide-laden wastewater is one step. The
pesticide waste disposal method is only complete when the pesticide phase is detoxified during SSF and the remaining matrix is considered to be non-hazardous.

4.2.2 Introduction

Safe pesticide use depends on correctly handling pesticides, which is why the label on each pesticide container dictates specific legally binding instructions. Also included on the pesticide label are instructions for the proper disposal of the container. Pesticide remaining in the original container, rinsate from the container, leftover amounts from application, and other rinsates all pose a threat to the health and stability of humans and the environment if released into the environment in an improper manner.

There are several options for the pesticide user to dispose of pesticide waste. The pesticide user can keep leftover pesticide to reuse it during a reapplication or apply it to boundary vegetation. Pesticide containers containing bulk or concentrated liquid pesticide should be triple-rinsed with tap water into application holding tanks before the container can be disposed of in landfills (Fitz, 1992). It is illegal to dump this rinsate because it is a hazardous waste as defined by the Resource Conservation and Recovery Act (RCRA), and the pesticide user is presented with a dilemma of how to dispose of the pesticide waste. Depending on the volume of the rinsate, the pesticide user must be concerned with the collection and storage of the pesticide waste before considering the disposal step. Pesticide users do have an option of paying disposal companies that will handle and dispose of the waste properly. Pesticide users can handle the waste themselves using the available technology and disposal end points, such as landfills where the law permits. Some pesticide dealers will offer to handle pesticide waste from pesticide users who buy from them (Allison, 1992). Of course, dealers still have to deal with the waste.

Several means for pesticide disposal exist, but these can be costly to the pesticide user generating the pesticide waste (Norwood, 1990). The isolation of the pesticide phase onto granular activated carbon and subsequent burial or incineration is a popular means for large scale disposal of pesticide waste (Krueger and Severn, 1984). Systems using granular activated carbon (GAC) work well for removing contaminants from wastewater, but the used activated carbon needs to be renewed or regenerated after use, which can be expensive. GAC originates from biobased products (e.g. coal, coconut shells, and peach pits), but research has been conducted using other biobased materials, such as peat moss,
chitin, and wood products (Coupal and Lalancette, 1976; Toller and Flaim, 1988; Mathan and Viraraghavan, 1989; Hetzel et al., 1989; Allen et al., 1994; Couillard et al., 1994) and chitosan fiber (Yoshida et al., 1991). Biobased sorbents are readily available, are a renewable resource, and are usually ready for use as a sorbent without much processing, which is required to manufacture activated carbon (Lehr, 1991). Peat moss and wood products are examples of biobased sorbents that contain mainly cellulose and lignin (Couillard, et al., 1994) and are called lignocellulosic materials. Lignocellulosic materials are especially good sorbents for removing polar contaminants from water because of the abundant polar functional groups present (Couillard et al., 1994).

An alternative method for pesticide disposal is being developed through a multidisciplinary research project at Virginia Tech (Hetzel et al., 1989; Mullins et al., 1992). The Virginia Tech pesticide waste disposal system illustrated in Figure 1 (Chapter 1, Introduction) is an effective and inexpensive means for the pesticide user to isolate pesticides from rinsate and then subsequently detoxify the pesticide phase through a combination of chemical and biological reactions, formation of bound residues, and volatilization. The initial disposal process involves mixing the pesticide-laden wastewater with lignocellulosic materials, such as peat moss and steam-exploded wood fibers. Isolation of the pesticide phase occurs by sorption to the lignocellulosic material. The majority of pesticides tested are removed during this batch sorption phase, but the wastewater is then passed through a secondary column filter containing lignocellulosic materials. Finally, the water is passed through filters of granular activated carbon (AC) to further reduce the pesticide concentration. The result is wastewater that has had the pesticide phase virtually removed, and therefore can be used safely for agricultural irrigation or for some other non-potable process. The lignocellulosic matrix containing the pesticide phase is placed in bioreactors and managed in a solid state fermentation process. The path of pesticide dissipation is very much dependent on the chemical composition of the pesticide. Pesticide degradation in the biologically active environment can occur abiotically, but because the solid state fermentation process is a highly biologically active process, pesticides can be degraded rapidly through biologically mediated reactions.

The purpose of these experiments was to compare the sorption capabilities of some lignocellulosic matrices with granular activated carbon (AC) and granular rubber in isolating chlorpyrifos from pesticide-laden wastewater. AC is a common sorbent for clarifying drinking water, as well as wastewater, with its large surface area and ability to
sorb a wide variety of compounds (Lehr, 1991). AC was used as a control for comparing how well chlorpyrifos was removed from wastewater by biobased sorbents. Ground rubber (R) was also compared for its sorption capabilities. Rubber from waste sources such as tires can be granulated and be used inexpensively as an alternative sorbent (Spencer, 1991). Lignocellulosic materials are readily available and are less expensive by weight than activated carbon or other synthetic adsorbents (Mullins et al., 1993). The lignocellulosic materials studied were peat moss (PM) and steam-exploded wood fibers (SEW). The second part of the Virginia Tech pesticide waste disposal system involves lignocellulosic material in a solid state fermentation process to degrade the isolated pesticide. Having the waste pesticide phase already isolated on lignocellulosic material prior to the degradation stage is sensible in the two part disposal process.

Emulsifiable concentrates (EC) are the predominant pesticide formulation because of the EC’s ability to increase the pesticide’s solubility and also to improve coverage on the waxy surface of plants (Ware, 1994). However, the stable emulsion formed becomes a barrier to sorption of the active ingredient in a pesticide disposal process. It has been demonstrated (Judge et al., 1989) that an emulsion is prohibitive to sorption of the active ingredient during batch adsorption. However, once the emulsion was disrupted or demulsified, sorption of the active ingredient increased. Effective demulsification was achieved by the addition of Ca(OH)₂ to the emulsifiable concentrate.

In addition to comparing sorbents for sorption capabilities, the effect on demulsification by adding Ca(OH)₂ to the emulsion was studied to determine if the sorption of chlorpyrifos improved. Also examined was the effect that a sorbent without the addition of Ca(OH)₂ had in disrupting the integrity of an emulsion and whether or not the sorption of chlorpyrifos would be improved. It was believed that the emulsion integrity would be disrupted by sorption of the surfactant chemicals and inert molecules. Partial to complete disruption of the emulsion should improve the sorption of chlorpyrifos to the sorbents that were studied.

The effect of filtering the wastewater before and after demulsification was also examined. It was believed that filtering would remove the suspended particulate phase that had originated from the lignocellulosic materials. The suspended particulates could have a considerable amount of chlorpyrifos associated with them, therefore, removing that phase would further reduce the pesticide concentration.

To compare the sorption capabilities of PM, SEW, R, and AC, and the effect that each treatment had in improving chlorpyrifos sorption, batch adsorption experiments were
performed. The initial part of the pesticide disposal process described by Hetzel et al. (1989) involves batch adsorption of the pesticide waste with a sorbent phase. The experiments described here were designed to result in adsorption isotherms, which are useful tools to compare sorbents and treatments. Adsorption isotherms consist of graphically comparing the aqueous concentration \( (C_e) \) of the analyte versus the sorbed phase of the analyte being tested \( (q_a) \) after an equilibrium between the two phases has been established. The determination of adsorption isotherms are conducted in aqueous systems with only a single sorbent phase and the pure analyte. The concentration of the analyte in water is limited by its water solubility and any adsorption to a sorbent results in a decrease in the aqueous concentration. In the case with emulsified solution of Dursban® 4E, the concentration of chlorpyrifos in the aqueous phase can be magnitudes greater than the water solubility of chlorpyrifos (approximately 2 \( \mu \)g/mL). For clarity the concentration of chlorpyrifos will be presented as an apparent concentration in each phase (aqueous concentration, \( C_a \); sorbent concentration, \( q_a \)).

An adsorption isotherm can be determined in two different experimental ways. Both ways involve having samples with varying ratios of the initial aqueous analyte concentration to the sorbent amount. One way of varying the aqueous analyte concentration to the sorbent ratio involves keeping the sorbent amount constant while varying the initial solution concentration of the analyte. This method is typical of constructing adsorption isotherms for analytes and soil. The other way of constructing adsorption isotherms is typical for comparing sorbents in the removal of compounds from wastewater (Bernardin, 1985). This other way involves keeping the initial analyte concentration constant while varying the sorbent amount. The latter method was used in this study because it was difficult to vary the concentration of chlorpyrifos in solution without drastically altering the state of the emulsion.

Assumptions for the correct use of an isotherm are that an equilibrium is established between adsorption and desorption processes, that there is no appreciable degradation, and that the concentration of the compound in the aqueous phase is not affected by suspended particles or colloids (Bernardin, 1985; Weber, 1985). Suspended particles or colloidal structures can give an artificially greater aqueous concentration, resulting in an inaccurate isotherm (Tye et al., 1996). It was believed that suspended particles and an intact emulsion would be present after equilibrium in the treatments with no filtering or no demulsification. The presence of the suspended particulates with associated chlorpyrifos would violate a criterion made for interpreting isotherms correctly.
Therefore, the primary comparison of all of the data was done by analysis of variance (ANOVA) of the aqueous chlorpyrifos concentrations between all of the sorbents (AC, R, SEW, and PM) and the different treatments (DF, DNF, NDF, NN). Secondarily, it was believed that the examination of adsorption isotherms from the filtered treatments (DF and NDF) for all four sorbents was important in understanding the sorption behavior.

The use of adsorption isotherms for comparing sorbents and their sorption qualities is usually based on one of two formats. One format for comparing isotherms relies on making general and non-statistical inferences on sorption and sorbent quality based on the shape of the untransformed isotherms and location and slope of the transformed isotherms in relation to each other. The shape of the isotherm can be important in determining the nature of the sorption processes (Weber, 1972; Giles and Easton, 1968). The different shapes of the isotherms shown in Figure 5 (modified from Weber, 1972) can indicate which sorbent is better for removal of an analyte from wastewater. The Type II or linear isotherm is indicative of a constant partition of the analyte between the aqueous phase and sorbent phase over the course of the isotherm. The Type I isotherm is considered to be favorable in comparison to the Type II (linear isotherm) or the Type III isotherm, displaying unfavorable adsorption (Figure 5). The Type I isotherm displays similar q_e values at the lower initial concentrations, whereas the Type III isotherm has a steeper decline in q_e values resulting in similar aqueous concentrations (C_e). In other terms, it takes more sorbent to remove the same amount of analyte from the aqueous phase, which translates to a sorbent with differing affinities or efficiencies for removing an analyte from the aqueous phase.

The second format for comparing isotherms involves comparing adsorption isotherms in a more rigorous way, which is accomplished by explaining the adsorption isotherms by a mathematical model. Modeling the data in such way that it becomes linear is favorable for comparing isotherms. Most mathematical models of adsorption isotherms result in a linear relationship of the data. If the relationship between the aqueous and sorbed concentrations is not linear, the use of a model describing non-linear sorption behavior is simply the linear model \( q_e = K C_e \), where \( q_e \) is the amount of the analyte per amount of sorbent at equilibrium, \( C_e \) is the concentration of the analyte at equilibrium, and \( K \) is the isotherm coefficient or slope. The models for non-linear isotherms describe the data by mechanistic or empirical relationships resulting in a linear relationship. Common
Figure 5  Shapes of Adsorption Isotherms And Sorption Behavior
Modified from Weber, 1972
non-linear models are Langmuir, Brunauer, Emmett, and Teller (BET), and Freundlich (Weber, 1985). The shape of the original isotherm can indicate which model might be more appropriate, but primarily it is the best fitted model that should be used. Figure 6 illustrates some common adsorption isotherm shapes that are linear when modeled by the Langmuir, BET, and Freundlich models.

The Langmuir adsorption isotherm (Langmuir, 1918) was one of the first attempts to explain adsorption phenomena with gases (Weber, 1985). The assumptions are that the energy of adsorption is constant and independent of surface coverage. The model is based on a monolayer of sorbed molecules. At equilibrium in solid-liquid systems, the Langmuir isotherm model is explained as:

\[
q_e = \frac{QbC_e}{1 + bC_e} \quad [\text{Equation 1}]
\]

where, \(q_e\) = the amount of analyte adsorbed per unit weight of sorbent at equilibrium

\(Q\) = is the concentration of analyte/sorbent when complete coverage occurs

\(b\) = an adsorption coefficient

\(C_e\) = the aqueous analyte concentration at equilibrium

In its linear form the Langmuir adsorption isotherm becomes:

\[
\frac{1}{q_e} = \frac{1}{Q} + \frac{1}{bQC_e} \quad [\text{Equation 2}]
\]

A plot of \(1/q_e\) vs. \(1/C_e\) giving a straight line will have a y-intercept of \(1/Q\) and a slope of \(1/bQ\).

The Brunauer, Emmett, and Tyler (BET) adsorption isotherm (Brunauer et al., 1938) was derived as an extension of the Langmuir adsorption isotherm model, which assumes that subsequent layers of molecules upon others that have been already adsorbed can form before a monolayer of adsorbed molecules is complete (Weber, 1985). The BET adsorption isotherm model is described as follows:

\[
q_e = \frac{BQC_e}{(C_e - C_s)[1 + (B-1)(C_e/C_s)]} \quad [\text{Equation 3}]
\]

where, \(q_e\) = the amount of analyte adsorbed per unit weight of sorbent at equilibrium

\(Q\) = is the concentration of analyte/sorbent when complete coverage occurs

\(B\) = an adsorption coefficient describing the energy of adsorption

\(C_e\) = the aqueous analyte concentration at equilibrium

\(C_s\) = the saturation concentration (solubility limit)
Figure 6  Nonlinear Models for Adsorption Isotherms

\( q_e \) is the amount of analyte sorbed per unit weight of sorbent at equilibrium.

\( C_e \) is the aqueous concentration of the analyte at equilibrium.

\( Q \) is the concentration of analyte/sorbent when complete coverage occurs.

\( C_s \) is the saturation concentration (solubility limit).

\( B \) is an adsorption coefficient describing the energy of adsorption.

\( n \) is an adsorption coefficient relative to adsorption intensity.

\( K_F \) is the Freundlich adsorption coefficient relative to adsorption capacity.
In its linear form the BET adsorption isotherm becomes:
\[ \frac{C_e}{(C_e - C_s)}q_e = \frac{1}{BQ} + \left[\frac{(B-1)/BQ}{C_e/C_s}\right] \]  
[Equation 4]

A plot of \( \frac{C_e}{(C_e - C_s)}q_e \) vs \( C_e/C_s \) giving a straight line will have a y-intercept of \( 1/BQ \) and a slope of \( (B-1)/BQ \).

The Freundlich adsorption isotherm model (Freundlich, 1926) is one that is commonly used to explain the sorption behavior of pesticides. The model takes into account multilayering of compounds sorbed, which may better explain what sorption processes are actually occurring (Weber, 1985). The Freundlich adsorption isotherm is an empirical relationship and is represented by the following equation:

\[ q_e = K_F C_e^{1/n} \]  
[Equation 5]

where, \( q_e \) = the amount of analyte adsorbed per unit weight of sorbent at equilibrium
\( K_F \) = the Freundlich adsorption coefficient relative to adsorption capacity
\( 1/n \) = an adsorption coefficient relative to adsorption intensity
\( C_e \) = the aqueous analyte concentration at equilibrium

In its linear form the Freundlich adsorption isotherm becomes:
\[ \ln q_e = \ln K_F + \frac{1}{n} (\ln C_e) \]  
[Equation 6]

A plot of \( \ln q_e \) vs \( \ln C_e \) giving a straight line will have a y-intercept of \( \ln K_F \) and a slope of \( 1/n \).

All of the linear forms of the different adsorption isotherms models and the corresponding slopes, intercepts, and the position on the graph in relation to the origin can be used to compare different sorbents to other sorbents. It is common to compare intercept values of parallel sorbents’ isotherms, or compare slopes of different sorbents’ isotherms. Usually, the greater the y-intercept value between isotherms confers better sorption capacity of a sorbent (Weber, 1985). Secondly, to compare isotherms based on the \( K_F \) value or y-intercept, the lines being compared should be parallel. Inferences can be made on whether or not isotherms are parallel or \( K_F \) values are different, but any serious comparison should involve statistics. As with any mathematical model explaining some phenomenon, the data being examined should fit the model well, otherwise, a different
model may better explain the data. Correlation coefficients or $r^2$ values give a good indication of how well data fit to a linear model, however, in statistical terms, a lack of fit test (LOF) should also be performed on the regressed data to determine if the data fit the model (Ott, 1988).

All of the linear forms of the different adsorption isotherms models and the corresponding slopes, intercepts, and the position on the graph in relation to the origin can be used to compare different sorbents to other sorbents. The comparison is based on the sorbents' ability to remove compounds from solution, efficiency of adsorption, and relative economy of using a particular sorbent. The closer an isotherm is to the abscissa and the farther it is away from the ordinate, the better its sorption qualities are (Figure 7).

4.2.3 Materials and Methods

4.2.3.1 Materials

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) formulated as Dursban® 4E was used in the Freundlich sorption isotherm determination. Analytical standards of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) that were greater than 99% pure were supplied by DowElanco (Indianapolis, IN). Unless otherwise specified, chemicals were reagent grade and solvents used were at least pesticide grade; these were purchased mainly from Fisher Scientific Inc. (Atlanta, GA).

The sorbents used during this study were supplied from various sources and are listed below. Canadian sphagnum peat moss with its origin in North Carolina was purchased from Blacksburg Feed & Seed (Blacksburg, VA). The steam-exploled wood (SEW) was supplied by the Virginia Tech Biobased Materials Center (Blacksburg, VA). The steam-explosion process described by Overend and Chornet (1987) involved a Masonite® process gun. The SEW originated from yellow poplar (Liriodendron tulipifera L.). The granular rubber used as a sorbent was from Neoprene® butyl rubber stoppers (Fisher Scientific Inc.). All matrices were ground and homogenized in a Wiley mill and passed through a 1 mm screen.
Figure 7 Comparison of Sorbents Using Freundlich Adsorption Isotherms
4.2.3.2 Methodology and Treatments

Batch Sorption Experiment

A batch sorption experiment was performed with various sorbents and chlorpyrifos formulated as an emulsion with Dursban® 4E. Transferred into triplicate 20 mL scintillation vials were 0.025, 0.1, 0.25, 0.4, 0.5, and 1.0 g amounts of each ground sorbent. The following summarizes the treatment for each set of samples before the extraction procedure:

1) samples demulsified and then filtered (DF)
2) samples demulsified but not filtered (DNF)
3) samples not demulsified but filtered (NDF)
4) samples not demulsified and not filtered (NN)

Samples that were to be demulsified had 300 mg of Ca(OH)₂ added to the 20 mL scintillation vials. A stock solution of Dursban® 4E was made such that the chlorpyrifos concentration was 5000 μg/g, which approximates some of the more concentrated solutions that would be encountered as pesticide waste (Hetzel et al. 1989). The resulting emulsion was stirred constantly with a magnetic stirbar and stirplate. Ten mL aliquots of the Dursban® 4E emulsion were added to all scintillation vials, including 18 vials with no sorbent (controls). All of the actual samples were mixed with a vortex mixer for one and two minutes, shaken on a shaker table for six hours, and then allowed to remain undisturbed for 18 hours.

Control samples involving the Dursban® 4E emulsion without sorbent were made for comparing the effects of filtering the solution and with or without adding Ca(OH)₂. The control samples included applying the initial Dursban® 4E emulsion to triplicate vials at the same time as the samples and treating the controls as follows:

1) No Ca(OH)₂ added: 48 hour mixing with no filtering
2) Addition of Ca(OH)₂: 48 hour mixing with no filtering
3) No Ca(OH)₂ added: 48 hour mixing followed by filtering
4) No Ca(OH)₂ added: 2 hour mixing, followed by settling period, and not filtered
5) Addition of Ca(OH)₂: 2 hour mixing, followed by settling period, and not filtered
6) Addition of Ca(OH)₂: 2 hour mixing, followed by settling period, then filtered
Due to time constraints, control samples were treated after 48 hours, therefore handled 24 hours after the actual samples with sorbents. Those controls designated to be mixed until sampled (controls 1 through 3) remained undisturbed for approximately 5 minutes prior to filtering or taking direct aliquots for determination of the concentration of chlorpyrifos.

Samples designated to be filtered were done so by decanting the 10 mL of solution solutions through a Buchner funnel with pre-wetted (H$_2$O) glass filters (2.0 μm pore size. The 10 mL of solution were decanted in a way to transfer the liquid portion while leaving as much of the sorbent behind. The filtrate was collected into a second 20 mL scintillation vial placed under the Buchner funnel inside a side-arm Erlenmeyer flask.

Aliquots (100 μL) of all actual samples and controls were then transferred to 9.9 mL of hexane in 20 mL glass scintillation vials (first 1/100 dilution) and mixed with a vortex mixer. The 100 μL aliquots were taken directly from overlying solution in the original scintillation vial for the unfiltered samples and from the second vial for the filtered samples. The vials containing the aliquot and hexane were capped and mixed by vortex mixer several times over one hour. Aliquots (100 μL) of each of the partitioned samples were placed into secondary 20 mL scintillation vials containing 9.9 mL hexane (second 1/100 dilution). The controls were additionally diluted by transferring 50 μL of the second dilution into 5 mL hexane (third 1/100).

Aliquots (0.25 mL) of the hexane/batch sorbent extract or the filtrate were diluted to 20 mL hexane for pesticide analyses. Additional sample preparation for pesticide analyses included addition of three, 20 mL (1/20, v/v, hexane/acetone) volumes followed by the addition of 2 or 3 drops of Keeper's solution (2% paraffin oil in hexane).

Column Sorption Experiment

A simple column experiment was performed where chlorpyrifos formulated as an emulsion of Dursban® 4E was passed through a 50 mL buret consisting of a layer of granular rubber on top of a layer of steam-exploded wood (SEW). Ten mL of SEW with 25 mL of methanol was poured into a 50 mL glass buret that had 0.25 cm of 0.25 mm glass beads on the bottom. Methanol was passed through the column to settle the SEW. A similar 10 mL portion of a granular rubber/methanol slurry was carefully poured on top of the SEW layer. Approximately 2 L of distilled H$_2$O were passed through the column to remove the methanol, wash the sorbent, and condition the column. A 10 mL aliquot of an emulsion of Dursban® 4E containing approximately 2500 μg/g chlorpyrifos was poured
onto the column. Two different 25 mL portions of H2O were passed through the column and the effluent collected separately for chromatographic analysis. The flow rate through the column was approximately 1 mL per minute (the total amount processed in approximately one hour was 60 mL). One mL aliquots of each 25 mL effluent portions were placed into 20 mL scintillation vials followed by 10 mL of hexane. The samples at this point were handled in a similar manner as were the batch sorption samples.

**Chromatographic Analysis**

The hexane partition from the batch and column sorption experiments was concentrated under nitrogen to a known volume for chromatographic analysis. The hexane partition from the column sorption experiment was taken directly for chromatographic analysis. Each sample was injected (2 to 5 μL) into a Tracor 540 gas chromatograph with a 6 ft glass column (14 inch I.D.) packed with Supelcoport® 100-120 mesh (1.5 - 1.95%, SP2250 - SP2401) equipped with an electron capture detector. Instrument temperatures were: injection port, 235 °C; column, 190 °C; detector, 350 °C; and carrier gas flow rate, 40 mL/min N2. The dead time of the column was approximately 0.2 minutes determined as the time it took a unretained compound (acetone) to pass through the column. The retention time for chlorpyrifos was generally 1.1 minutes. Extracts of matrix blanks without chlorpyrifos were analyzed and there were no interfering peaks with chlorpyrifos. The concentration of chlorpyrifos determined in the aqueous phase and the sorbent phase is defined as an apparent concentration. The concentration of chlorpyrifos in solution where an emulsion is intact versus a demulsified solution would be different. The concentration of chlorpyrifos in an emulsifiable concentrate can be many times greater than its water solubility of 2 μg/mL.

**Statistical Analysis**

The primary comparison of the various treatments (DF, DNF, NDF, NN) and sorbents (AC, R, SEW, PM) to remove chlorpyrifos from the aqueous phase was done by an analysis of variance (ANOVA) of the apparent chlorpyrifos concentrations between the various treatments. The ANOVA tests were done by using the general linear models (GLM) procedure using software developed by Statistical Analysis Service (SAS, 1985).

An analysis of covariance (ANCOVA) was chosen as the statistical test for comparing the Freundlich adsorption isotherms from the filtered treatments (DF and NDF)
and the four sorbents examined. ANCOVA is an appropriate statistical test to compare different treatments that have linear relationships. The assumptions of ANCOVA are that the regression relationship is linear and that the regressions being compared are parallel (Ott, 1988). The level of significance was chosen to be an $\alpha = 0.05$ for both the ANOVA and ANCOVA analyses. How well the data fit the Freundlich model through linear regression was examined by reporting the correlation coefficient or $r^2$ values from the least squares fit and also by a lack of fit test (LOF). The ANCOVA and linear regression were performed using the statistical software package called Statmost (version 2; DataMost Corporation). The LOF test was done by partitioning the sum of squares due to error (SSE) from the ANCOVA results into a pure experimental portion ($SS_{exp}$) and also a lack of fit portion ($SS_{Lack}$) shown as the following equation: $SSE = SS_{exp} + SS_{Lack}$ (Ott, 1988). The $SS_{Lack}$ was determined from the difference of the SSE taken from the ANCOVA results and the $SS_{exp}$ determined as the $\sum(Y_{ij} - Y_i)^2$ from the data in a computer spreadsheet software package (Excel, version 5, Microsoft®), where $Y_{ij}$ = each datum for the $j^{th}$ observation ($j = 3$ samples per treatment) at the $i^{th}$ level of the independent variable ('i' = 6 sorbent amounts); and $Y_i$ = the mean of data at the $i^{th}$ level. The means square due the LOF part ($MS_{Lack}/MS_{exp}$) was compared to the appropriate $F$ statistic to determine if a significant LOF existed (generally, $F_{crit} = (df_1 = 3; \ df_2 = 6) = 4.76$ (Ott, 1988). $MS_{Lack} = SS_{Lack}/n_i$, where $n_i = 3$, and $MS_{exp} = SS_{exp}/df$, where $df = (i - 1)(j - 1) = 10$.

4.2.4 Results and Discussion

During the batch sorption experiments, the sorption phase occurred for 24 hours. The sorption processes in all samples were subjected to the same mixing condition (first 6 hours) and settling conditions (final 18 hours) at approximately $22^\circ$C for 24 hours. No preliminary experiments were done to determine if an equilibrium was established within the 24 hour period. In an extension of the batch adsorption isotherm experiments including one treatment (SEW, filtered and demulsified), the chlorpyrifos concentration remained unchanged after an additional 24 hours were allowed to pass. A conclusion could not be made as to whether or not an equilibrium was established in the situations where the emulsion remained intact.
The initial chlorpyrifos concentration for all samples was determined to be $4332 \pm 323 \, \mu g/mL$ (mean ± standard error of the mean or SEM; $n = 8$). The results from the various control samples without sorbent are summarized in Figure 8. The controls were filtered or extracted an additional 24 hours after the actual samples due to time constraints. To keep the controls as similar as possible to the samples, all controls were mixed again after the first 24 hours and then allowed to remain undisturbed for 18 hours. Controls with Ca(OH)$_2$ were repeated to avoid hydrolysis at the higher pH using extra controls that had not contained Ca(OH)$_2$. As shown in Figure 8, the concentration of chlorpyrifos of the Dursban$^\text{®}$ 4E control samples (mixed and not filtered) that were mixed for an additional 24 hours was similar at $4189 \pm 217 \, \mu g/mL$ to the concentration determined at 24 hours. Filtering the mixed Dursban$^\text{®}$ 4E control samples resulted in a reduction of the chlorpyrifos to 81% of the original amount applied (Figure 8). There was some adsorption of chlorpyrifos to the filtering unit; however, in preliminary experiments involving similar initial conditions, it was concluded that adsorption to the wetted glass filters was not a problem. It was not clear why there were contrasting results. Chlorpyrifos is a non-polar compound (water solubility is approximately 2 $\mu g/mL$) and adsorption to the filtering components (glass filter and Buchner funnel) would seem likely. Factors such as sedimentation of the emulsion after the filtration and prior to taking aliquots for extraction may have reduced the chlorpyrifos that was recovered. It should be understood that in this study, filtration may have involved chlorpyrifos adsorption to the filter, in addition to removing any chlorpyrifos associated with suspended particulate material. It was believed that comparing filtered with non-filtered samples was a valid comparison even with an apparent problem with chlorpyrifos adsorption to the filter unit, since the same adsorption phenomenon may be observed in the actual field situation. Removal of chlorpyrifos by whatever means is an objective for the pesticide waste disposal process.

On the other hand, it is not advisable to use the sorption data from the filtered versus non-filtered samples in adsorption isotherms. One criterion for performing valid adsorption isotherms is ensuring that the mass balance is accounted for the aqueous and solid sorbent phases (Weber, 1985). In conducting an adsorption isotherm, if the analyte adsorbs to the filter unit in amounts approaching 20%, as indicated by the controls in this study, then the amount of chlorpyrifos in the sorbent phase would be erroneously calculated for the adsorption isotherm.
Figure 8 Results of Controls (no Sorbent Present):
Aqueous Chlorpyrifos Concentration after Several Treatments

Vertical lines on bars represent the standard error of the mean (n = 3)

Treatment ¹: initial concentration of chlorpyrifos was 4332 µg/mL
Ca(OH)₂ Added ² (300 mg in 10 mL emulsifiable concentrate)
Mixed ³: All mixed samples mixed for 48 hours
Filtered ⁴ (samples filtered after 48 hours with 2 µm glass filter)
Allowed to settle ⁵ for 46 hours after mixing for 2 hours
As concluded in Project I of this dissertation, the chlorpyrifos concentration should be directly correlated to the concentration of the emulsion. One objective of this study was to reduce the chlorpyrifos in solution by disrupting the emulsion. An emulsified solution such as the Dursban® 4E emulsion that is cloudy and white indicates that the emulsion is intact (Becher, P., 1973). The emulsion formed from Dursban® 4E is defined as a macroemulsion because the emulsion formed makes the solution turbid or cloudy, unlike a microemulsion that is clear when formed (Sharma and Shah, 1985). A completely disrupted or demulsified macroemulsion will be clear.

The emulsion in initial Dursban® 4E solution did not appear to be stable over time. The controls (settled and not filtered) became clearer by just leaving the solution undisturbed for 48 hours. The solutions in the controls left to settle remained cloudy and white, and there was some white material that had settled to the bottom of the vials. The settled material was likely excess surfactant or compounds involved with the emulsifiable concentrate. Related to the solution becoming clearer was a decrease in the concentration of chlorpyrifos in these controls to $1391 \pm 270 \, \mu g/mL$, which was 33% of the concentration in the initial solution ($4332 \, \mu g/mL$ chlorpyrifos) (Figure 8). A certain amount of surfactant per volume of water is required to establish micelles, defined as the critical micelle concentration (Becher, P., 1973). More surfactant can be added beyond the critical micelle concentration and the emulsion remains stable, however, the emulsion becomes unstable after a certain amount of surfactant has been added. This limit is defined as the hydrophile lipophile balance (HLB) (Becher, P., 1973). The HLB for Dursban® 4E was approximately the amount of the formulation required to result in $1391 \, \mu g/mL$ of chlorpyrifos. The label for Dursban® 4E states that the solution needs to be agitated and not left to settle. Solutions containing amounts of the formulated Dursban® 4E greater than the HLB are possible, but agitation is required to keep the emulsion homogeneous.

The solution in the demulsified controls remained cloudy and white, but was definitely clearer than those left undisturbed without Ca(OH)$_2$ added. The controls designated to mix for 48 hours were left undisturbed for approximately 5 minutes prior to either filtering or directly taking aliquots (not filtered) for chlorpyrifos determination. Even after 5 minutes of remaining undisturbed, solid white material had settled to the bottom of the vial, which were probably excess surfactants and undissolved Ca(OH)$_2$. There was decrease in the concentration of chlorpyrifos to $390 \pm 102 \, \mu g/mL$ (9% of the initial concentration). The pH of the emulsions with Ca(OH)$_2$ added were approximately
11. Hydrolysis of chlorpyrifos to 3,4,5-trichloro-2-pyridinol has been shown in solutions with alkaline pH (Macalady and Wolfe, 1983). The degree of hydrolysis of chlorpyrifos increases as the pH increases and the half-life of chlorpyrifos at pH 8 has been reported to be approximately 35 days (DowElanco fact sheet). Based on the half-life information, hydrolysis of chlorpyrifos would have occurred in the controls with Ca(OH)₂ added and may have accounted for 10 to 20% of the decrease in the concentration of chlorpyrifos. However, hydrolysis would not have accounted for the additional decrease in the concentration of chlorpyrifos. The majority of the decrease in the concentration was due to the disruption of the emulsion promoted by the addition of Ca(OH)₂ and the subsequent sorption.

An additional decrease in the aqueous concentration of chlorpyrifos to 2% (89 ± 39 µg/mL) occurred by allowing the “demulsified” control samples Ca(OH)₂ added to settle for 18 hours before taking aliquots for chlorpyrifos determination (Figure 8). Filtering this solution decreased the chlorpyrifos concentration even further to 1% (47 ± 40 µg/mL) of the original amount of chlorpyrifos.

It was concluded from the results involving the controls that the concentration of chlorpyrifos formulated as Dursban® 4E emulsion was affectively decreased by a combination of allowing solutions in excess of the HLB to remain undisturbed, adding Ca(OH)₂ to the solution, and filtering the solution. As expected, the addition of Ca(OH)₂ had a profound effect on disrupting the emulsion and subsequently conferring a decrease in the concentration of chlorpyrifos. However, complete demulsification did not occur in the controls as evident by the solution remaining cloudy and white. The amount of Dursban® 4E in solution was above the critical micelle concentration after the addition of Ca(OH)₂.

Complete demulsification of the Dursban® 4E was achieved during this study, but only in situations where sorbents were present. There was a wide range of solution clarity in samples containing sorbents with or without the addition of Ca(OH)₂. Figure 9 illustrates the general clarity of the solutions after the various treatments in samples containing AC, R, SEW, or PM. For all treatments, a trend in solution clarity occurred in the solutions directly proportional to the amount of sorbent. Complete demulsification was defined as a clear solution achieved by either the addition of 300 mg Ca(OH)₂ and a certain amount of sorbent or by the sorbent itself.

The addition of Ca(OH)₂ did not always result in complete demulsification. The solutions with Ca(OH)₂ added that were not completely demulsified displayed varying
Figure 9  Graphical Representation of Solution Clarity after Various Treatments to a Dursban 4E Emulsion

- Continuum of cloudiness of solution directly proportional to darkness of square
- DF = Demulsified then Filtered
- DNF = Demulsified & Not Filtered
- NDF = Not Demulsified but Filtered
- NN = Not Demulsified & Not Filtered
degrees of the cloudiness. Solutions that were not clear were cloudy and white for the AC and R sorbents and cloudy and yellow for the lignocellulosic sorbents. Complete demulsification was most pronounced in the AC and R sorbents (Figure 9). All AC and R solutions with Ca(OH)₂ added at sorbent amounts of 0.1 g and below were cloudy and white. Solutions at AC amounts above 0.1 g were clear. The SEW solutions with Ca(OH)₂ added at the 0.5 g and 1.0 g sorbent amounts were clear. The only PM solution with Ca(OH)₂ added that was clear was the 1.0 g sorbent amount after filtration. The clear solutions from the AC and R were colorless. The clear solutions from the SEW and PM were yellow, which was probably due to pigments leaching from the lignocellulosic material.

The sorbents did have the capacity to promote demulsification without the presence of Ca(OH)₂. Clear solutions were achieved in R samples without Ca(OH)₂ in sorbent amounts greater than 0.1 g (Figure 9). Clear solutions were also achieved in the AC, SEW, and the PM samples without Ca(OH)₂, but only at the 1 g sorbent amounts. The demulsification by the sorbent alone was most likely due the affinity for the surfactants and capacity of the sorbent to disrupt the integrity of the emulsion below the critical micelle concentration by sorption of the surfactants. The R sorbent was the most effective sorbent examined at demulsification without adding Ca(OH)₂. The R sorbent is predominantly hydrophobic in nature, which confers a strong affinity for the non-polar portion of the surfactant compounds. Micelle structure may be better disrupted when the inner nonpolar portion of the micelle involving the surfactants is what is sorbed.

The conclusion that a sorbent alone caused demulsification was examined further in a simple column sorption experiment. It was shown in Project I that an intact emulsion was a barrier to sorption of the emulsified pesticide. Once the emulsion was disrupted, sorption of the pesticide improved. As described in the Materials and Methods above (Section 4.2.3), a column composed of a layer of granular rubber overlying steam-exploded wood fibers was effective in not only completely demulsifying the Dursban® 4E emulsion, but also was quite effective in removing chlorpyrifos from solution. The 10 mL emulsion containing approximately 2500 μg/mL was a cloudy white emulsion as it was poured onto the column, but the two different consecutive effluents were clear and contained only ppb concentrations of chlorpyrifos. The concentration of chlorpyrifos was determined to be approximately 110 ppb (ng/mL) in the first 25 mL of effluent collected after 10 mL of approximately 2500 μg/mL chlorpyrifos in an emulsion formulated as Dursban® 4E had been put onto the column. The second 25 mL portion contained 120
ppb chlorpyrifos. Further additions of distilled H₂O were not done. It was concluded from this simple experiment that passing a solution of pesticide formulated as an emulsion through a column constructed as above could effectively demulsify a sorb the pesticide portion.

The effect that emulsion stability and the various treatments and sorbent had on clarifying or demulsifying the Dursban® 4E emulsion is important, but it is important to correlate those observations with chlorpyrifos removal from solution. The apparent concentration of chlorpyrifos in the aqueous phase (Cₐ) and sorbent phase (qₐ) for each sorbent type and the four different treatments are presented in Tables 6 through 13. The sorption data are presented in two different ways for comparisons at each sorbent weight. The data are separated as tables by sorbent in Tables 6 through 9 and the data are separated by treatment in Tables 10 through 13. In both comparative aspects (by sorbent or by treatment), there were significant differences (ANOVA; P < 0.05) of apparent chlorpyrifos concentrations between different sorbents and also between the various treatments.

The percent of chlorpyrifos removed or sorbed from solution from all of the treatments and sorbents examined ranged from 69.3% (SEW; NDF; 0.025 g) to 99.5% (AC & R; DF; 1.00 g) (Table 6 through 9). The 69.3% amount of chlorpyrifos removed in the SEW samples (NDF, 0.025 g) compared with the 67% of the chlorpyrifos removed in the undisturbed controls (33% remaining in solution; Figure 8). As mentioned before, a considerable amount of chlorpyrifos removal from a Dursban® 4E emulsion originally made to 4332 µg/mL was achieved by just allowing the solution to remain undisturbed and the unstable portion of the emulsion to settle.

The addition of Ca(OH)₂ was the significantly more important treatment for improving the removal of chlorpyrifos from the aqueous phase in the AC and R samples (Tables 6 and 7) than in the SEW and PM samples (Tables 8 and 9). There were significant differences between the demulsified treatments (DF & DNF) and the treatments that were not demulsified (NDF & NN) in the AC and R sorbents at the majority of sorbent weights (Tables 6 and 7). It is likely that the improvement in the removal of chlorpyrifos from solution was related to demulsification improving sorption, however, some hydrolysis of chlorpyrifos may have occurred due to the presence of Ca(OH)₂.

There would have been some chlorpyrifos degradation due to hydrolysis at an approximate pH of 11, but after 24 hours hydrolysis would not have accounted for the large difference in the chlorpyrifos concentration observed between samples with or without Ca(OH)₂.
<table>
<thead>
<tr>
<th>Sorbent Amount (g)</th>
<th>Demulsified then Filtered (DF)</th>
<th>Demulsified not Filtered (DNF)</th>
<th>Not Demulsified but Filtered (NDF)</th>
<th>Not Demulsified &amp; Not Filtered (NNF)</th>
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<td>$C_a^2$</td>
<td>$\text{sem}^3$</td>
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<tr>
<td></td>
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$^1$Treatment: Demulsification prompted by addition of 0.3 g Ca(OH)$_2$ per 10 ml of Durbar® 4E

$^2$C$_a$ = apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 ml total volume)

$^3$sem = standard error of the mean (n = 3)

$^4$q$_a$ = apparent chlorpyrifos concentration in the solid phase (ppm x 10$^6$)

$^5$Percent sorbed = 100 x chlorpyrifos sorbed/total chlorpyrifos applied (total chlorpyrifos applied = 41.32 mg)

$^6$Multiple comparison results (Dunnett's Multiple Range Test for means). Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level.
### Table 7: Sorption Isotherm Results of Chlorpyrifos Formulated as Dursban® 4E (4331 ppm) and Granulated Rubber Subjected to Various Treatments

<table>
<thead>
<tr>
<th>Sorbent Amount (g)</th>
<th>Demulsified then Filtered (DF)</th>
<th>Demulsified not Filtered (DNF)</th>
<th>Not Demulsified but Filtered (NDF)</th>
<th>Not Demulsified &amp; not Filtered (NN)</th>
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</tr>
<tr>
<td>0.50</td>
<td>4.90</td>
<td>na</td>
<td>86.5</td>
<td>99.89</td>
</tr>
<tr>
<td>1.00</td>
<td>2.16</td>
<td>1.06</td>
<td>43.3</td>
<td>99.95</td>
</tr>
</tbody>
</table>

---

1. **Treatment**: Demulsification prompted by addition of 0.5g Ca(OH)$_2$ per 10 mL of Dursban® 4E
2. **$C_a$**: apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 mL total volume)
3. **sem**: standard error of the mean (n = 3)
4. **$q_a$**: apparent chlorpyrifos concentration in the solid phase (ppm x 10$^5$)
5. **Percent Sorbed = 100 x chlorpyrifos sorbed/total chlorpyrifos applied (total chlorpyrifos applied = 43.32 mg)
6. **Multiple comparison results (Duncan's Multiple Range Test for means)**: Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level.
Table 8  Sorption Isotherm Results of Chlordane Formulated in Durban® 4E (4331 ppm) and Steam-Exploded Wood Subjected to Various Treatments

<table>
<thead>
<tr>
<th>Sorbent Amount (g)</th>
<th>Demulsified then Filtered (DF)</th>
<th>Demulsified not Filtered (DNF)</th>
<th>Not Demulsified but Filtered (NDF)</th>
<th>Not Demulsified &amp; Not Filtered (NN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_a^2$</td>
<td>$q_a^4$</td>
<td>$C_a$ mg/g Sorbed</td>
<td>$C_a$</td>
</tr>
<tr>
<td></td>
<td>µg/mL</td>
<td>µg/mL</td>
<td></td>
<td>µg/mL</td>
</tr>
<tr>
<td>0.025</td>
<td>153.11</td>
<td>22.24</td>
<td>1671.1</td>
<td>96.46</td>
</tr>
<tr>
<td>0.10</td>
<td>259.42</td>
<td>25.46</td>
<td>4074.9</td>
<td>94.01</td>
</tr>
<tr>
<td>0.25</td>
<td>100.93</td>
<td>22.18</td>
<td>169.0</td>
<td>97.67</td>
</tr>
<tr>
<td>0.40</td>
<td>41.50</td>
<td>4.84</td>
<td>1072</td>
<td>99.04</td>
</tr>
<tr>
<td>0.50</td>
<td>43.95</td>
<td>6.19</td>
<td>1075</td>
<td>98.99</td>
</tr>
<tr>
<td>1.00</td>
<td>11.75</td>
<td>4.22</td>
<td>43.2</td>
<td>99.73</td>
</tr>
</tbody>
</table>

1Treatment: Demulsification prompted by addition of 0.3 g Ca(OH)$_2$ per 10 ml of Durban® 4E
2$C_a$ = apparent chlordane concentration in the aqueous phase (ppm in 10 ml total volume)
3sem = standard error of the mean (n = 3)
4$q_a$ = apparent chlordane concentration in the solid phase (ppm x 10$^6$)
5Percent sorbed = 100 x chlordane sorbed/total chlordane applied (total chlordane applied = 4332 mg)
6Multiple comparison results (Duncan's Multiple Range Test for means): Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level
Table 9  Sorption Isoterm Results of Chlorpyrifos Formulated as Dursban® 4E (4331 ppm) and Pest Moss Subjected to Various Treatments

<table>
<thead>
<tr>
<th>Sorbent Amount (g)</th>
<th>Demulsified then Filtered (DF)</th>
<th>Demulsified not Filtered (DNF)</th>
<th>Not Demulsified but Filtered (NDF)</th>
<th>Not Demulsified &amp; Not Filtered (NN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; sem&lt;sub&gt;m&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; sem&lt;sub&gt;m&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; sem&lt;sub&gt;m&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; sem&lt;sub&gt;m&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
</tr>
<tr>
<td>µg/mL.</td>
<td>µg/mL.</td>
<td>µg/mL.</td>
<td>µg/mL.</td>
<td>µg/mL.</td>
</tr>
<tr>
<td>0.025</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;2&lt;/sub&gt; m&lt;sub&gt;g&lt;/m/g Sorbed&lt;sub&gt;m&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Results&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Results&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Results&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Results&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.10</td>
<td>325.84 36.27 1599.6 92.84 A</td>
<td>326.59 31.30 1605.2 92.46 A</td>
<td>868.96 58.35 1383.6 79.94 B</td>
<td>770.90 58.71 1422.3 82.20 B</td>
</tr>
<tr>
<td>0.25</td>
<td>473.54 52.96 3846.6 89.02 AB</td>
<td>469.74 46.01 3542.0 91.20 A</td>
<td>638.26 35.23 369.6 85.26 BC</td>
<td>755.09 97.42 356.5 82.57 C</td>
</tr>
<tr>
<td>0.40</td>
<td>472.93 24.90 1019.4 94.15 A</td>
<td>548.28 63.77 151.0 87.34 A</td>
<td>426.58 12.15 156.3 90.15 A</td>
<td>468.81 26.66 154.5 89.18 A</td>
</tr>
<tr>
<td>0.50</td>
<td>497.38 37.85 82.8 90.59 A</td>
<td>314.05 19.84 80.4 92.75 D</td>
<td>239.33 23.60 102.3 94.47 A</td>
<td>281.84 19.47 101.2 93.49 A</td>
</tr>
<tr>
<td>1.00</td>
<td>59.70 9.12 42.7 98.62 A</td>
<td>273.53 10.04 40.6 93.68 C</td>
<td>62.37 3.74 42.7 98.56 A</td>
<td>86.30 2.71 42.5 98.01 B</td>
</tr>
</tbody>
</table>

<sup>1</sup>Treatment: Demulsification prompted by addition of 0.3 g Ca(OH)<sub>2</sub> per 10 mL of Dursban® 4E

<sup>2</sup>C<sub>m</sub> = apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 mL total volume)

<sup>3</sup>sem = standard error of the mean (n = 3)

<sup>4</sup>q<sub>m</sub> = apparent chlorpyrifos concentration in the solid phase (ppm x Y<sub>m</sub>)

<sup>5</sup>Percent Sorbed = 100 x chlorpyrifos sorbed/total chlorpyrifos applied (total chlorpyrifos applied = 43.32 mg)

<sup>6</sup>Multiple comparison results (Duncan's Multiple Range Test for means): Within a row (sorbent amount), results with the same letter are significantly similar at the 0.05 significance level
Table 10  Sorption Isotherm Results of Chlorpyrifos Formulated as Durban® 4E (413 ppm) and Various Sorbent Types After a Treatment of Demulsification Followed by Filtration

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Activated Carbon (AC)</th>
<th>Rubber (R)</th>
<th>Steam-Exploded Wood (SEW)</th>
<th>Peat Moss (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount (g)</td>
<td>C chase{superscript}2 chase{subscript} chase{superscript}m chase{superscript}3</td>
<td>( \bar{C} ) chase{subscript}s</td>
<td>( q_s^{4} ) Percent Multiple</td>
<td>C chase{superscript}2 chase{subscript} chase{superscript}m chase{superscript}3</td>
</tr>
<tr>
<td></td>
<td>( \mu g/mL )</td>
<td>( \mu g/mL )</td>
<td>( \mu g/mL )</td>
<td>( \mu g/mL )</td>
</tr>
<tr>
<td>0.025</td>
<td>97.05</td>
<td>3.66 1694.3 97.76</td>
<td>49.42</td>
<td>3.97 422.6 98.14</td>
</tr>
<tr>
<td>0.10</td>
<td>80.54</td>
<td>3.97 422.6 98.14</td>
<td>259.42</td>
<td>25.46 407.4 94.01</td>
</tr>
<tr>
<td>0.25</td>
<td>28.44</td>
<td>5.06 172.2 99.34</td>
<td>100.93</td>
<td>23.18 169.0 97.67</td>
</tr>
<tr>
<td>0.50</td>
<td>7.76 4.36 108.1 99.82</td>
<td>41.50</td>
<td>4.84 107.2 99.04</td>
<td>252.93</td>
</tr>
<tr>
<td>1.00</td>
<td>4.90 4.86 86.5 99.90</td>
<td>43.95</td>
<td>6.19 107.9 98.99</td>
<td>407.38</td>
</tr>
<tr>
<td></td>
<td>2.16 1.06 43.3 99.95</td>
<td>11.75</td>
<td>4.22 43.2 99.73</td>
<td>59.70</td>
</tr>
</tbody>
</table>

1 Demulsification prompted by addition of 0.3 g Ca(OH)\(_2\) per 10 mL of Durban® 4E
2 \( \bar{C} \) = apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 mL total volume)
3 SEM = standard error of the mean (n = 3)
4 \( q_s \) = apparent chlorpyrifos concentration in the solid phase (ppm x 10\(^{4}\))
5 Percent sorbed = 100 \times chlorpyrifos sorbed/total chlorpyrifos applied (total chlorpyrifos applied = 43.32 mg)
6 Multiple comparison results (Duncan's Multiple Range Test for means): Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level.
Table 11  Sorption Isotherm Results of Chlorpyrifos Formulated as Durban® 4E (4331 ppm) and Various Sorbent Types After a Treatment of Demulsification, but not Filtration

<table>
<thead>
<tr>
<th>Sorbent Type</th>
<th>Activated Carbon (AC)</th>
<th>Rubber (R)</th>
<th>Steam-Exploded Wood (SEW)</th>
<th>Peat Moss (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount (g)</td>
<td>C\textsubscript{a} \textsubscript{2} \textsuperscript{a}</td>
<td>C\textsubscript{a} \textsubscript{3} \textsuperscript{a}</td>
<td>C\textsubscript{a} \textsubscript{4} \textsuperscript{a}</td>
<td>C\textsubscript{a} \textsubscript{5} \textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>µg/mL</td>
<td>mg/g Sorbed</td>
<td>µg/mL</td>
<td>mg/g Sorbed</td>
</tr>
<tr>
<td>0.025</td>
<td>179.63</td>
<td>13.58</td>
<td>1664.3</td>
<td>96.06</td>
</tr>
<tr>
<td>0.10</td>
<td>71.87</td>
<td>5.97</td>
<td>426.0</td>
<td>98.34</td>
</tr>
<tr>
<td>0.35</td>
<td>42.95</td>
<td>8.70</td>
<td>171.5</td>
<td>90.01</td>
</tr>
<tr>
<td>0.40</td>
<td>14.23</td>
<td>2.48</td>
<td>107.9</td>
<td>99.67</td>
</tr>
<tr>
<td>0.50</td>
<td>11.80</td>
<td>3.41</td>
<td>86.4</td>
<td>99.73</td>
</tr>
<tr>
<td>1.00</td>
<td>3.50</td>
<td>0.82</td>
<td>43.3</td>
<td>99.92</td>
</tr>
</tbody>
</table>

1 Demulsification prompted by addition of 0.3 g Ca(OH)\textsubscript{2} per 10 mL of Durban® 4E
2 C\textsubscript{a} = apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 mL total volume)
3 \textsuperscript{a} = standard error of the mean (n = 3)
4 C\textsubscript{a} = apparent chlorpyrifos concentration in the solid phase (ppm x 10\textsuperscript{3})
5 Percent sorbed = 100 \times \frac{chlorpyrifos sorbed}{total chlorpyrifos applied} (total chlorpyrifos applied = 43.32 mg)
6 Multiple comparison results (Duncan’s Multiple Range Test for means): Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level.
<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Activated Carbon (AC)</th>
<th>Rubber (R)</th>
<th>Steam-Exploded Wood (SEW)</th>
<th>Peat Moss (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount (g)</td>
<td>C&lt;sub&gt;a&lt;/sub&gt;</td>
<td>SEM</td>
<td>q&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Percent</td>
</tr>
<tr>
<td>0.025</td>
<td>1232.1</td>
<td>1230.6</td>
<td>1239.7</td>
<td>71.55</td>
</tr>
<tr>
<td>0.10</td>
<td>806.40</td>
<td>27.38</td>
<td>343.5</td>
<td>79.29</td>
</tr>
<tr>
<td>0.25</td>
<td>578.35</td>
<td>42.66</td>
<td>150.1</td>
<td>86.64</td>
</tr>
<tr>
<td>0.40</td>
<td>206.17</td>
<td>11.65</td>
<td>103.3</td>
<td>95.38</td>
</tr>
<tr>
<td>0.50</td>
<td>120.37</td>
<td>2.70</td>
<td>84.2</td>
<td>97.22</td>
</tr>
<tr>
<td>1.00</td>
<td>20.07</td>
<td>1.89</td>
<td>43.0</td>
<td>99.33</td>
</tr>
</tbody>
</table>

1 C<sub>a</sub> = apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 mL total volume)
2 SEM = standard error of the mean (n = 3)
3 q<sub>a</sub> = apparent chlorpyrifos concentration in the solid phase (ppm x 10<sup>5</sup>)
4 Percent sorbed = 100 x chlorpyrifos sorbed/total chlorpyrifos applied (total chlorpyrifos applied = 43.32 mg)
5 Multiple comparison results: results within a sorbent amount (row) with similar letters are significantly similar at the 0.05 significance level using Duncan's Multiple Range Test for means
6 Multiple comparison results (Duncan's Multiple Range Test for means): Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level.
Table 13  Sorption Isotherm Results of Chlorpyrifos Formulated as Durban® 4E (4331 ppm) and Various Sorbent Types with neither Demulsification or Filtration

<table>
<thead>
<tr>
<th>Sorbent Amount (g)</th>
<th>Activated Carbon (AC)</th>
<th>Rubber (R)</th>
<th>Steam-Exploded Wood (SEW)</th>
<th>Pest Moss (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₄є₁ µg/mL C₄є² mg/g Sorbed² Comparison</td>
<td>C₄є₁ µg/mL C₄є² mg/g Sorbed² Comparison</td>
<td>C₄є₁ µg/mL C₄є² mg/g Sorbed² Comparison</td>
<td>C₄є₁ µg/mL C₄є² mg/g Sorbed² Comparison</td>
</tr>
<tr>
<td></td>
<td>Results⁵</td>
<td>Results⁵</td>
<td>Results⁵</td>
<td>Results⁵</td>
</tr>
<tr>
<td>0.25</td>
<td>794.90 34.24 1415.8 81.65 A</td>
<td>639.73 33.09 1475.7 85.23 A</td>
<td>1079.0 71.26 1297.2 75.09 B</td>
<td>770.90 58.71 1422.3 82.20 A</td>
</tr>
<tr>
<td>0.10</td>
<td>789.23 7.47 354.0 81.78 B</td>
<td>256.45 50.33 406.4 94.08 A</td>
<td>812.83 83.34 351.6 81.23 B</td>
<td>755.00 97.42 356.5 82.57 B</td>
</tr>
<tr>
<td>0.25</td>
<td>675.03 12.23 146.2 84.40 B</td>
<td>175.79 15.76 166.3 95.94 A</td>
<td>587.49 122.47 148.6 86.44 B</td>
<td>468.81 26.60 154.5 89.18 B</td>
</tr>
<tr>
<td>0.40</td>
<td>297.73 17.00 100.9 93.13 B</td>
<td>106.91 6.75 195.7 97.53 A</td>
<td>338.06 29.01 99.8 92.19 B</td>
<td>281.84 19.47 101.2 93.49 B</td>
</tr>
<tr>
<td>0.50</td>
<td>197.97 7.54 82.6 95.45 B</td>
<td>91.41 6.21 106.9 99.89 A</td>
<td>347.54 27.48 79.6 91.98 C</td>
<td>314.11 34.13 79.1 91.36 C</td>
</tr>
<tr>
<td>1.00</td>
<td>88.00 7.18 42.5 97.97 B</td>
<td>36.81 3.27 43.3 99.16 A</td>
<td>68.08 17.32 42.7 98.66 AD</td>
<td>85.30 2.71 42.5 98.01 B</td>
</tr>
</tbody>
</table>

¹C₄є = apparent chlorpyrifos concentration in the aqueous phase (ppm in 10 mL total volume)
²sem = standard error of the mean (n = 3)
³C₄є = apparent chlorpyrifos concentration in the solid phase (ppm x 10³)
⁴Percent sorbed = 100 x chlorpyrifos sorbed/total chlorpyrifos applied (total chlorpyrifos applied = 43.32 mg)
⁵Multiple comparison results (Duncan’s Multiple Range Test for means). Within a row (sorbent amount), results with the same letters are significantly similar at the 0.05 significance level
For example, the chlorpyrifos concentrations in the 1 g AC solutions with and without Ca(OH)$_2$ were approximately 3 µg/mL and between 30 to 90 µg/mL, respectively (Table 6). In both cases, over 99% of the chlorpyrifos was not in solution. As discussed before, hydrolysis may have accounted for 10 to 20% of the decrease in the chlorpyrifos concentration in the samples with Ca(OH)$_2$, but not the entire observed 99% removal.

The solutions in the AC samples at 1 g with and without Ca(OH)$_2$ were both clear, indicating that complete demulsification occurred in both situations. It already has been demonstrated that the addition of Ca(OH)$_2$ promotes emulsion disruption and a subsequent decrease in chlorpyrifos. In those 1 g AC samples without Ca(OH)$_2$, the demulsification occurred by adsorption of surfactants comprising the emulsion. Concentrations of chlorpyrifos in completely demulsified solution of Dursban® 4E should be less than the water solubility of 2 µg/mL without the existence of an emulsion enabling concentrations of chlorpyrifos to be greater than the water solubility. However, concentrations of chlorpyrifos were greater than 2 µg/mL in samples with seemingly completely demulsified solutions. A plausible reason for this observation involves the surfactants and the critical micelle concentration. A clear solution has been defined in this study as one that is completely demulsified, however, a clear solution does not necessarily mean that the solution is devoid of micelles or surfactants. A clear solution may have had a concentration of micelles near the critical micelle concentration and some chlorpyrifos associated with the micelles, resulting in a chlorpyrifos concentration greater than its water solubility. The bulk of the decrease in the concentration of chlorpyrifos was achieved by adding Ca(OH)$_2$. Further surfactant sorption from the aqueous phase occurred by sorption to the sorbents. Some chlorpyrifos would have been associated with the portion of the surfactants that remained in the water phase in an unstructured manner, but at a surfactant concentration below the critical micelle concentration.

There was not an improvement in the removal of chlorpyrifos from solution by filtering the AC or R samples (Tables 6 and 7). At all but the 0.4 and 0.5 g sorbent amounts, filtering did not significantly improve chlorpyrifos removal. Filtering did have a pronounced and significant effect on improving the clarity of the solutions and on chlorpyrifos removal in the lignocellulosic samples (SEW & PM). As the SEW and PM sorbent amounts increased, filtering was nearly as effective as demulsification at influencing chlorpyrifos removal. Filtering samples was expected to increase chlorpyrifos removal from solution with the lignocellulosic sorbents, but not necessarily with the AC or R sorbents. In previous research with SEW and PM (Project I), suspended particulates
had been observed in the aqueous phase after allowing the batch adsorption to remain undisturbed. It was believed that by removing the suspended particulate phase through filtration, the overall removal of chlorpyrifos would be improved. On the other hand, it was thought that the AC and R sorbents would settle quicker and to a greater extent than the lignocellulosic sorbents because of differences in the densities of the sorbents. The activated carbon sank to the bottom in water and in the emulsion. The rubber sorbent was denser than water, but it floated in water due to hydrophobic surface of the rubber being repelled by water. However, the rubber particles sank when the Dursban® 4E was added to the water. The reason for this is that the nonpolar portion of the surfactant associates with the nonpolar surface of the rubber and the polar portion of the surfactant orients outwards into the water. This creates a surfactant-rubber particle that has a polar outer surface that allows association with the water. The AC and R sorbents settled to a greater extent than the lignocellulosic sorbents and filtering the aqueous phase resulted in an only marginal improvement after demulsification.

The aqueous and sorbent chlorpyrifos concentration data from the filtered treatments (DF and NDF) summarized in Tables 6 through 9 were plotted as adsorption isotherms, as well as Freundlich adsorption isotherms (Figures 10 through 17). The shape of adsorption isotherms can give information concerning the sorption behavior and is illustrated in Figure 5 (Weber, 1972). All filtered adsorption isotherms were indicative of the unfavorable Type III isotherm, but the portion of the isotherms involving the 0.25 to 1.0 g sorbent amounts appears to have displayed a favorable or Type 1 isotherm for most of the treatments (Figures 10 through 17). There was no clear reason why the isotherms of the two lignocellulosic sorbents (SEW and PM) with Ca(OH)₂ added for demulsification had the shape that they did (Figures 14 and 16). The sorbent amount of 0.025 g in both sorbents resulted in a lower apparent chlorpyrifos concentration in the aqueous phase than with the sorbent amount of 0.1 g. The isotherms addressing the lignocellulosic sorbents without Ca(OH)₂ present displayed the characteristic Type III curve (Figures 7 and 8). One possible explanation is that a colloidal suspension was formed in the samples with sorbent amounts 0.1 g or greater. Most colloidal suspensions are not removed by filtration due to the 1 nm to 1 μm colloidal particle size (Gregory, 1989). The colloidal suspension may have involved the appropriate ratio of Ca(OH)₂, the lignocellulosic sorbent, and the emulsion chemicals. At the appropriate ratios, both divalent cations (Gregory, 1989) and suspended particulates (Levine and Sanford, 1985) can stabilize emulsions. Even with the Ca(OH)₂ present to promote demulsification, the
Figure 10  Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Activated Carbon Treated by Demulsification Followed by Filtration
Figure 11  Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Activated Carbon Treated by Filtration (No Demulsification)
Adsorption Isotherm

```
Treatment
Granular Rubber
Demulsified then Filtered

Apparent Chlorpyrifos Concentration in Solid Phase (q_a) (mg chlorpyrifos/g sorbent)
```

```
Apparent Chlorpyrifos Concentration in the Aqueous Phase (C_a) (μg/mL)
```

Freundlich Adsorption Isotherm

```
Treatment
Granular Rubber
Demulsified then Filtered

K_F = 54.1; \( r^2 = 0.660 \)
```

```
Log Scale: Apparent Chlorpyrifos Concentration in Solid Phase (q_a) (mg chlorpyrifos/g sorbent)
```

```
Log Scale: Apparent Chlorpyrifos Concentration in the Aqueous Phase (C_a) (μg/mL)
```

Vertical and horizontal bars are the standard error of the mean and are shown where they exceed the symbol

Figure 12 Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Granular Rubber Treated by Demulsification Followed by Filtration
Figure 13  Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Granular Rubber Treated by Filtration (No Demulsification)
Vertical and horizontal bars are the standard error of the mean and are shown where they exceed the symbol.

Figure 14 Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Steam-Exploded Wood Treated by Demulsification Followed by Filtration
Figure 15 Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Steam-Exploded Wood Treated by Filtration (No Demulsification)
Figure 16  Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Peat Moss Treated by Demulsification Followed by Filtration
Vertical and horizontal bars are the standard error of the mean and are shown where they exceed the symbol.

Figure 17 Adsorption Isotherms for Chlorpyrifos Formulated as Dursban® 4E and Peat Moss Treated by Filtration (No Demulsification)
solutions were cloudy and colored in the lignocellulosic samples with less than 0.5 g sorbent. A colloidal suspension involving soluble lignocellulosic material would have likely had some chlorpyrifos associated with it, thus increasing the apparent chlorpyrifos concentration. Other evidence supports this explanation. The chlorpyrifos concentrations in solutions with PM that had Ca(OH)$_2$ added were slightly greater or similar compared to PM solutions without Ca(OH)$_2$ (Table 9). These results were contrary to the results involving the AC or R (Tables 6 and 7), where a decrease in the concentration of chlorpyrifos occurred with the addition of Ca(OH)$_2$. In similar and related studies involving an emulsifiable concentrate of metolachlor (Dual 8E), Hutchinson et al. (1993) found similar results of higher metolachlor concentrations in solutions with Ca(OH)$_2$ and SEW or PM compared with similar solutions without Ca(OH)$_2$ added. They suggested that soluble humic substance may have increased with the higher pH brought about with the addition of Ca(OH)$_2$. Greater humic substance concentrations would confer greater metolachlor concentrations due to that portion of metolachlor that would associate with the humic substances in solution. The same phenomenon of greater concentrations was observed in this study with the PM samples, but was not observed with the SEW samples. The ratio of Ca(OH)$_2$ and sorbent to the volume of the formulated material was different than this study (Hutchinson et al., 1993: 33.3 mL solution/g sorbent and 0.4 g Ca(OH)$_2$/g sorbent; this study: 10 to 400 mL solution/g sorbent and 0.3 to 1 g Ca(OH)$_2$/g sorbent).

Colloidal suspensions are much like an emulsion when it comes to stability (Gregory, 1989). When one component is reduced below a critical concentration, the interactions between the solution, colloid particles, and any stabilizing ions will change and the suspension may separate. The addition of SEW or PM at amounts of 0.025 g or less may have actually stabilized the emulsion, but as more sorbent was added, the stability of the colloidal suspension would have been diminished due to sorption of the surfactant portion of the colloidal suspension. The apparent aqueous concentration of chlorpyrifos would have also decreased because there was more sorbent present. The colloidal stabilization would not necessarily have occurred in the controls or samples with only the lignocellulosic sorbents, since either component would have been missing and unable to stabilize the colloidal suspension.

The results of the Freundlich adsorption isotherms are summarized in Table 14. The $r^2$ values ranged from 0.394 in the PM FD to 0.919 in the R NDF. One criterion that had to be met for the ANCOVA analysis to be valid was a linear association between the two variables (aqueous vs sorbent concentration) (Ott, 1988). The $r^2$ values are a general
Table 14  Summary of Freundlich Adsorption Isotherms

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Treatment</th>
<th>$K_F^2$</th>
<th>$r^2$</th>
<th>Slope</th>
<th>F value$^4$</th>
<th>LOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated Carbon</td>
<td>FD</td>
<td>23.8</td>
<td>0.775</td>
<td>0.82</td>
<td>15</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>FND</td>
<td>2.5</td>
<td>0.793</td>
<td>0.75</td>
<td>1,223</td>
<td>Y</td>
</tr>
<tr>
<td>Granular Rubber</td>
<td>FD</td>
<td>54.1</td>
<td>0.661</td>
<td>0.49</td>
<td>519,385</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>FND</td>
<td>0.76</td>
<td>0.919</td>
<td>1.1</td>
<td>1,388</td>
<td>Y</td>
</tr>
<tr>
<td>Steam Exploded</td>
<td>FD</td>
<td>4.2</td>
<td>0.638</td>
<td>0.91</td>
<td>51,397</td>
<td>Y</td>
</tr>
<tr>
<td>Wood</td>
<td>FND</td>
<td>2.1</td>
<td>0.867</td>
<td>0.80</td>
<td>22,146</td>
<td>Y</td>
</tr>
<tr>
<td>Peat Moss</td>
<td>FD</td>
<td>0.69</td>
<td>0.394</td>
<td>1.0</td>
<td>15,585</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>FND</td>
<td>4.8</td>
<td>0.814</td>
<td>1.2</td>
<td>5,579</td>
<td>Y</td>
</tr>
</tbody>
</table>

Lack of Fit Test (LOF)$^3$  

**Treatment$^1$:** Demulsification (D) was promoted by the addition of 300 mg Ca(OH)$_2$/10 mL.  
Filtered (F) samples occurred after demulsification (2 μm glass filter)  

**$K_F^2$:** Freundlich adsorption isotherm coefficient  
Lack of Fit Test (LOF)$^3$ was performed to determine if the data statistically fit the linear model  
Details of LOF test are described in the text  

**F value$^4$:** $F_{critical}$ (3, 10) ($α=0.05$) = 3.71
indication of how well the data fit a model - in this case a linear model - however, they are not used as statistical test for the fit of the data to the model (Ott, 1988). The lack of fit (LOF) test used was the appropriate statistical test, and in all cases there was a significant lack of fit to the linear Freundlich adsorption model (Table 14). From these results, it was concluded that the criteria for comparing data by ANCOVA methods were not met and, therefore, the Freundlich adsorption isotherm model did not adequately model the sorption data. The data were also applied to the Langmuir, BET, and linear models without an improvement on the fit (results not shown).

The isotherms plotted in the log-log scale were nonlinear, which would have largely contributed to the poor correlation (r² values) and significant LOF. In all treatments, the isotherm portion involving the lowest two or three sorbent amounts (0.025, 0.1, and 0.25g) deviated from the rest of isotherm involving sorbent amounts greater than 0.25 g. This deviation may have been a result of having the surfactant portion of the initial Dursban® 4E solution compete with the chlorpyrifos for adsorption sites or effectively reduce the amount of chlorpyrifos that becomes associated with the sorbent. This was suggested by Willems and Berry (1993) as the reason for nonlinearity in their adsorption isotherms in a similar study with metolachlor formulated as Dual 8E at similar initial concentrations. Hutchinson et al., (1993) performed similar adsorption studies with Dual 8E, but the initial concentrations of metolachlor were a magnitude less than those in this study or the study performed by Willems and Berry (1993). The Freundlich model may explain sorption behavior for chlorpyrifos formulated as Dursban® 4E only at lower concentrations where sorption deviations would not occur.

Another major reason for the significant LOF may have been due to the variability of the aqueous concentration at each sorbent (Tables 6 through 9). Historically, Freundlich adsorption isotherms and the resulting coefficients (Kf, and 1/n) are determined by plotting the solution concentrations at equilibrium on the x-axis and the related sorbed amount on the y-axis. Both variables appear to be dependent variables, dependent on the amount of a particular sorbent and not each other. Either variable is not a truly independent variable, even though it could be argued that the amount sorbed is calculated based on the sorbent amount, therefore, it is an independent variable.

Traditionally, to perform regression linear regression analyses with the least squares method, it is proper to plot the independent variable on the x-axis and the data of dependent variable on the y-axis (Campbell, 1989). It is accurate to define statistical analyses performed on Freundlich adsorption isotherms as correlation analyses, rather than
regression analyses, because of the two dependent variables. It is a fine point to discern, but correlation analyses involve comparing how well two variables, in whatever dependency on each other, associatively correlate to each other (Campbell, 1989). The difference is in the assumptions made. In correlation analyses, it is assumed both variables display a normal distribution, whereas, in regression analyses, it is assumed that only the dependent variable is normally distributed (Campbell, 1989). The derivation of the statistical tests used in correlation and regression analyses are the same in most situations, therefore, the use of the least squares method for determining the correlation coefficient of the isotherms was appropriate in this study. The least squares method can be used for either correlation analyses that interpret associative relationships or regression analyses that interpret causative relationships (Birkes and Dodge, 1993). The consideration to make when correlating two dependent variables is which one is to be displayed as the y-values. Both the least squares method and the LOF test calculate the variability of the y values for a given x value. In this study, the variability observed for either variable was predominantly that of the apparent solution concentrations (C_a) (Figure 10 -17), which is the x-axis variable. A LOF test was performed on the data that had been switched to the other axis, which moved the variability observed (C_a) as y-axis values. The sum of squares as a result from pure experimental error were reduced, but there was still a significant LOF (results not shown) for all of the Freundlich adsorption isotherms from the filtered treatments (Figures 10 through 17). After further consideration and a more rigorous examination of the Freundlich adsorption isotherms, it was concluded that the data could not be explained by the Freundlich adsorption isotherm model. A different model should be developed in the future to better explain the sorption phenomenon observed with the sorption of two components - the chlorpyrifos and the surfactants - in solution.

4.2.5 Conclusion

Adding Ca(OH)_2 and filtering a solution of Dursban® 4E formulated to simulate typical pesticide waste encountered by the pesticide user were shown to be the best overall treatments to remove chlorpyrifos from the aqueous phase. It was demonstrated that in enough quantity, all sorbents without adding Ca(OH)_2 could effectively disrupt the Dursban® 4E emulsion, thus removing the barrier for sorption of chlorpyrifos. Filtering the samples after treatment was demonstrated to be more effective for increasing the
removal of chlorpyrifos in samples with the lignocellulosic materials than samples with the AC or R sorbents.

When the various sorbents are compared to one another in each of the various treatments, the R sorbent was significantly better or similar than the other sorbents in its ability to remove chlorpyrifos from the aqueous phase (Tables 10 through 13). Activated carbon was nearly as effective as the rubber in the demulsified treated samples (DF and DNF), but not as effective as the R in the non-demulsified treated samples. This was probably due to the R sorbent’s better hydrophobic nature. It is interesting that the SEW was significantly similar to both the R and the AC sorbents in removing chlorpyrifos from the aqueous phase in the DF treatment (Table 10). This is important to consider when choosing which sorbent would be best for a batch type of sorption in the pesticide waste disposal method proposed by Mullins et al. (1992) at Virginia Tech. The cost of the sorbents is a consideration in designing the disposal system. Activated carbon is fairly expensive at approximately $2/lb. The lignocellulosic materials are less costly at $0.07/lb for SEW and $0.05/lb for peat moss (Hutchinson et al., 1993). Rubber may be less costly, considering that it is a waste product from old used tires. Granularization of used tires is a common process used to incorporate the granular rubber in asphalt for road construction (Spencer, 1991). The lignocellulosic materials are renewable resources and actually are part of the second portion of the pesticide waste disposal method, which involves solid state fermentation of lignocellulosic materials and the isolated pesticide waste.

In the pesticide wastewater disposal system developed by Mullins et al. (1992), the objective is to remove the pesticide portion from the wastewater and then degrade the isolated pesticide portion by solid state fermentation (SSF) (Figure 1; Introduction). Secondly, but not less important, the objective is to have the resulting water phase virtually free of pesticides, so that it can be discarded as a nonhazardous water source and used in a non-potable fashion, such as irrigation. The goal of isolating the chlorpyrifos portion of the concentrated emulsion for subsequent degradation was achieved by using the lignocellulosic sorbents. Even though the lignocellulosic sorbents SEW and PM were not as effective as AC or R for removing chlorpyrifos from chlorpyrifos-laden wastewater initially at 4332 μg/g, the bulk (>95%) of chlorpyrifos was removed from the aqueous phase (approximately 153 μg/g) with use rates exceeding 4 g PM/L and 2.5 g SEW/L of formulated Dursban® 4E. The chlorpyrifos concentration was approximately 12 μg/g with 100 g SEW/L of formulated Dursban® 4E. Even with AC as the sorbent at use rates of
100 g sorbent/L of formulated Dursban® 4E, there was still approximately 2 μg/g chlorpyrifos left in solution after sorption had occurred. In both cases, it is likely that the water phase would require further steps to remove the chlorpyrifos before the water could be considered non-hazardous. A secondary systems of sorption and filtration is currently included in the pesticide waste disposal system being developed (Mullins et al., 1992). The additional pesticide left in solution after a primary batch sorption phase with SEW or PM is further removed by secondary filtration steps. The resulting lignocellulosic sorbents from a batch sorption and secondary filtration is then directly transferred to the SSF bioreactors for detoxification of the isolated pesticide phase.

The isolation or sorption step of the pesticide waste disposal system should be thought of as two distinct parts: an initial batch sorption phase that removes the bulk of pesticide for direct transfer to the degradation stage, and a secondary phase, which removes essentially all of the pesticide phase from the wastewater, so that the water is nonhazardous. It is possible that with further research the batch sorption process could be replaced with a column sorption process with lignocellulosic materials being used as the sorbent. The column sorption experiment performed in this study resulted in an effluent containing approximately 100 ppb chlorpyrifos. The low concentration of chlorpyrifos indicated that the overall pesticide removal was better than the batch sorption process. The column sorption process provides a combination of demulsification, sorption, and filtration of pesticide-laden wastewater.

In the future, comparison of sorbents for their sorption capabilities in the pesticide waste disposal system being developed at Virginia Tech should be done using direct statistical comparisons or using an adsorption model that correctly explains the sorption of the pesticide. In this study, the presence of an emulsion and the surfactants in a Dursban® 4E formulation interfered with the sorption of chlorpyrifos from solution. Because of the interference, the Freundlich adsorption isotherm model, as well as other adsorption models, did not explain the sorption data. There is an opportunity to develop models for this non-linear sorption behavior in a system that has several factors not generally covered by the conventional adsorption models. These factors include the differences of sorption of the analyte in solutions with an emulsion present and in solutions after complete demulsification. It is not clear to what extent the presence of surfactants in a solution formulated as an emulsifiable concentrate interferes with the sorption of the pesticide in solution, but further research could provide some interesting information for modeling a multicomponent sorption process.
4.3 PROJECT III  The Fate of Atrazine and Chlorpyrifos in Several Field-Scale Composting Systems Designed to Degrade Pesticide Waste

4.3.1 Abstract

Different field scale bioreactor structures and compost management practices were studied to optimize biological activity and pesticide detoxification. Increased microbial activity in compost has been associated with elevated compost temperatures, therefore measures were taken to increase temperatures. It was thought that higher temperatures conferring greater microbial activity and greater overall chemicals reactions would subsequently increase the detoxification of atrazine, chlorpyrifos, and malathion placed into the bioreactors.

Peat moss and steam-exploled wood based bioreactors in 189 Rubbermaid® containers had temperatures between 35 and 40 °C after amendments with corn meal and urea as a nitrogen source. Dissipation time for 50% parent compound disappearance (DT₅₀) for atrazine and chlorpyrifos in this bioreactor situation were 99 and 136 days from initial concentrations of approximately 8947 and 22,000 µg/g, respectively.

Temperatures between 45 and 50 °C were achieved in another experiment involving the 189 L bioreactor units. The higher temperatures were attained partly because of better bioreactor aeration and partly from the addition of vegetable oil. The vegetable oil was deep frying oil waste from a fast food restaurant and it appeared to have better coverage in the bioreactor matrix than did the corn meal. The better coverage of the vegetable oil and the fact that lipids yield approximately twice as much energy as similar amounts of carbohydrates were reasons for the higher temperatures.

Three different bioreactor structures were constructed. Polyurethane plastic bags designed for composting with aeration holes were tested as an alternative composting container for degrading pesticides. Another bioreactor structure tested was an approximate 1 m diameter cylindrical bioreactor unit enclosed with galvanized steel mesh fencing. The other bioreactor structure was a 1 m³ enclosed structure with a galvanized steel mesh bottom for air to enter the bioreactor and two vents on top of the unit to trap pesticides venting from the bioreactor on polyurethane foam traps (PUF's). Small cloth bags (10 by 10 cm) containing biobased materials and pesticides were placed into the actual bagged and cylindrical bioreactor structures, which facilitated sampling. Similarly,
galvanized steel mesh cylinders containing biobased materials with pesticide were placed into the cubic meter enclosed bioreactor units.

Temperatures exceeded 50 °C in the plastic bags, but the highest temperatures achieved during this study were between 65 to 70 °C in the cylindrical and cubic meter bioreactor units. Evidently, it is difficult to attain these higher temperatures in smaller bioreactors due to the loss of heat. Malathion degraded quickly in these hotter bioreactors with DT$_{50}$ values approximately a week in all bioreactors. Initial concentrations in the bagged and cylindrical bioreactors were approximately 2425, 1827, and 1023 μg/g for atrazine, chlorpyrifos, and malathion. The dissipation of atrazine in the cylindrical bioreactor with a DT$_{50}$ value of 14 days was only slightly better than the plastic bag bioreactor with a DT$_{50}$ value of 17 days. However, dissipation of atrazine initially at 3487 μg/g was much quicker in the cubic meter bioreactor with a DT$_{50}$ value of 2 days. The chlorpyrifos DT$_{50}$ values for the bagged and cylindrical bioreactor were 50 and 20 days, respectively. It was interesting that chlorpyrifos in the cubic meter bioreactor had a DT$_{50}$ value of 59 days, which was similar to the bagged bioreactor. It is important to note that the pesticide dissipation was slowed in the cubic meter bioreactor due to a decrease in bioreactor temperatures during the winter.

Chlorpyrifos is a more volatile compound (2.53 mPa; 25 °C) than atrazine (0.037 mPa; 20 °C) and it was detected in higher amounts than atrazine. Malathion has a vapor pressure of (1.03 mPa; 25 °C), but because of the quick degradation in the bioreactor, malathion was not detected in the PUF's after the first two weeks. The total amounts of all three pesticides recovered from the PUF volatile traps did not exceed 1 mg over the 13 day measurement period. Evidently, the pesticides, especially chlorpyrifos volatilized from the hotter region of the bioreactor only to condense in the cooler outer region. Volatilization of the pesticides outside the compost did not appear to be a major route of pesticide dissipation in the cubic meter bioreactor. However, it may become important when pesticide is incorporated in the entire bioreactor, not just placing the sampling units inside the bioreactor.

Concluded from these experiments are the following management practices recommended for maximal microbial activity as indicated by high bioreactor temperatures. As mentioned before, the bioreactor volume should not be less than one cubic meter. Bulking materials, such as peanut hulls, should be added to the bioreactor to help promote good aeration. A supplemental nitrogen source (chicken manure) is required in this composting system which has a high C:N ratio (> 100:1) from primarily peat moss and
steam-exploded wood. Grass clippings with a 12 to 15 C:N ratio can be added to the bioreactor and are a good source of energy and nitrogen. It was found that vegetable oil added to the bioreactor was a good supplemental source of energy promoting increased bioreactor temperatures. Finally, it is important to keep the bioreactor hydrated and mix or turn the bioreactor approximately every 2 weeks to redistribute the bioreactor materials. If the previous suggestions are followed, temperatures should exceed 50 ºC, and a healthy hot bioreactor appears to promote the best conditions for pesticide dissipation.

4.3.2 Introduction

How to dispose of pesticide waste is a question that anyone who uses pesticides must ask. Of course, pesticide waste can be as simple as the can of aerosol left over after applying the contents for flea control in a home, or as voluminous as thousands of liters of pesticide-laden wastewater from a large agricultural operation. It is illegal to merely "dump" pesticides. If there is improper pesticide use, inadequate containment of waste, or improper disposal of waste pesticides, contamination of groundwater or contamination of streams or rivers may occur.

There are several suggestions for pesticide users to dispose of their pesticide waste. The best method of pesticide waste disposal is the practice of eliminating or greatly reducing the amount of waste generated. Most applicators will apply all of the pesticide made on or in the area of application. Advances in container and packaging have also greatly reduced the volume of pesticide waste. Some pesticides are sold in bulk containers, which can be reclaimed by the distributor for reuse and/or proper disposal (Allison, 1992; Hansen and Palmer, 1992). Some distributors do have a system of disposing of their customers' pesticide waste containers, however, the pesticide-laden rinsewater after cleaning equipment still poses a problem for disposal. It is proper to collect this wastewater in a temporary containment for future disposal. A common means of disposal for the wastewater is to sorb the pesticide phase to granular activated carbon (GAC) and then have the GAC combusted or buried in specially designed landfills for hazardous waste.

Mullins et al. (1989, 1990, 1992) are developing an alternative system for disposal of pesticide-laden wastewater, which is illustrated in Figure 1 (Chapter 1, Introduction).
The first part of the disposal process involves isolating the pesticide phase from the pesticide-laden wastewater in a batch sorption process with lignocellulosic materials, such as peat moss, steam-exploded wood, or steam-exploded peanut hulls. The batch adsorption isolates the majority of the pesticide phase of the wastewater. Pesticide is further removed by passing the water leftover after the batch sorption through a column of lignocellulosic materials. As a final step, the resulting water can be passed through tertiary filters containing granular activated carbon or other specially designed materials (Berry et al., unpublished data). At this point, the water is virtually devoid of pesticides and can be used for non-potable agricultural uses.

The second portion of the pesticide disposal process (Mullins et al., 1992) involves taking the pesticide portion isolated on the biobased sorbent to specially designed bioreactors. The contaminated sorbent matrix is incubated for a period of time under very active aerobic biological conditions, and the pesticide dissipates through chemical and biological reactions.

The research performed and presented in this Section was designed to study the parameters affecting biological activity in a composting situation. It was the objective to optimize those parameters by designing bioreactors and by improving composting management techniques. The intended result of achieving an optimized biologically active situation is increased pesticide dissipation.

Mullins et al. (1989) used 0.75 m³ watertight bioreactors for research on pesticide disposal. The dissipation of diazinon in these bioreactors using peat moss as the composting matrix was dramatic. Diazinon concentrations initially as high as 32,000 µg/g in these pits degraded to approximately 6 µg/g after 18 weeks with peak bioreactor temperatures greater than 50 °C. Research with these bioreactors demonstrated that diazinon degradation was enhanced by increasing microbial activity through amendment with fertilizer for growth processes and corn meal, which is an easily metabolized energy source.

Compost temperature is considered to be a good indication of microbial activity in compost. Golueke (1972) and Finstein et al. (1986) have stated that compost temperature is probably the best and easiest parameter to monitor microbial activity and the rate of decomposition. The optimum composting temperatures for degrading vegetative material have been reported to be between 55 and 60 °C (MacGregor et al., 1981) or 50 °C in another report (Schulze, 1961; Miller, 1993). All researchers in compost science agree that internal compost temperatures should be 35 °C or greater for good decomposition.
Composting temperatures near 70 °C and above are detrimental to most thermophilic bacteria and fungi because enzyme systems are affected and growth is inhibited (Mathur, 1991; Biddlestone et al., 1987). However, for municipal solid waste (MSW) composting, compost temperatures should be 60 °C or above to kill pathogenic microorganisms (Biddlestone et al., 1987).

A temperature profile typical of compost is illustrated in Figure 18. Temperatures generally increase when the compost is first put together. With an input of vegetative material in a newly constructed compost, bacterial and fungal populations grow and temperatures rise. As the easily metabolized organic compounds (i.e. carbohydrates, amino acids, lipids) in the compost are used for catabolism and anabolism, the microbial populations that thrived at higher temperatures (thermophilic microorganisms) begin to decline. Even though the thermophilic microorganisms constituted the largest and most active organisms during the composting sequence, metabolism in the compost does not cease. In fact, species diversity increases in the compost as it matures. As the temperature decreases, several species can compete for the remaining energy in the compost (Figure 18). What remains after thermophilic microorganism metabolism are the larger and more difficult organic compounds to metabolize. Difficulty to metabolize is defined as the increasing energy used by an organism (i.e. specialized enzyme systems) to metabolize a compound, which results in a lower overall yield of carbon and energy for catabolic and anabolic cellular processes. Lignocellulosic material is composed of large and complicated molecules of cellulose, holocellulose, hemicelluloses, proteins, and lignins, which can provide energy to organisms, but the organisms using the lignocellulosic material as an energy source have specific enzyme systems and cometabolism is common. A consortium of organisms is involved in decomposing the remaining organic material. Invertebrates move into the compost and their activity of feeding fragments the organic material, creating a larger surface area for other organisms (other invertebrates, fungi, bacteria) to digest the organic material (Figure 18). There is a succession of organisms in the compost which change as the compost matures and all that is left is the most difficult molecules to metabolize. However, an input of new vegetative material into the compost system starts the ecological succession all over again. It is on the succession of organisms involved with the decomposition of vegetative
Figure 18 Compost Temperature Profile & Ecological Succession
material that composting is based. It is also the basis for detoxifying pesticides put into a composting system designed as a pesticide waste disposal system.

Several experiments were performed at the Virginia Tech Turf Grass Farm Research Center located in Blacksburg, VA, as well as in the laboratory, which involved different bioreactor structures and compost management techniques. It was hypothesized that by increasing biological activity as indicated by heightened bioreactor temperatures, the rate of pesticide dissipation could be increased. Some of the bioreactor experiments were performed without pesticides to study what effects bioreactor structure and management practices had on increasing biological activity. Other experiments were performed to study the effect that bioreactor structure and management practices had on increasing pesticide degradation. The results of these experiments, as well as conclusions, are summarized in the following Sections.

4.3.2 Materials and Methods

4.3.2.1 Materials

The Ciba -Geigy Corporation (Greensboro, NC) supplied formulated atrazine as AAtrex® 4L, and the following analytical-grade reference standards (purity > 98%): 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-s-triazine [atrazine or ATZ], and 2-hydroxy-4-ethylamino-6-isopropylamino-1,3,5-s-triazine [hydroxyatrazine or HYA]. Supplied by DowElanco (Indianapolis, IN) were formulated chlorpyrifos as Dursban® 4E and the following analytical-grade reference standards (purity > 98%): O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate [chlorpyrifos], and 3,5,6-trichloro-2-pyridinol [TCP].

The steam-exploded products used as bioreactor matrices during these experiments were supplied by the Virginia Tech Biobased Materials Center (Blacksburg, VA). The steam-explosion process described by Overend and Chornet (1987) involved a Masonite® process gun. Yellow poplar (Liriodendron tulipifera L.) was used to produce the steam-exploded wood fibers (SEW), and Birdsong Inc. (Petersburg, VA) supplied the peanut hulls which were used in the steam-explosion process and also were used in their original form during these experiments as a bioreactor bulking agent.
Canadian sphagnum peat moss, shredded pine bark with its origin in North Carolina, Ca(OH)₂ in the form of 'burnt lime', and ground corn meal were purchased from Blacksburg Feed and Seed (Blacksburg, VA). Dried chicken manure was supplied by Virginia Tech Department of Poultry Science. Waste vegetable oil left after deep frying was supplied by Burger King Corporation (Blacksburg, VA). Leaves and fresh grass clippings were supplied by Grounds and Maintenance at Virginia Tech.

Unless otherwise specified, chemicals were reagent grade and solvents used were at least of HPLC grade; these were purchased primarily from Fisher Scientific (Atlanta, GA).

4.3.2.2 Methodology

The following methods are those common methods used for all of the experiments conducted. Any method unique to a particular experiment is outlined in the Experimental Design Section below pertaining to the respective experiment.

Bioreactor Temperatures

Bioreactors' temperatures were either recorded manually with either a digital thermometer (Omega Instruments), which had an approximate 20 cm temperature probe attached, or were recorded electronically with up to 16 different temperature probes, and the data were stored for later retrieval (data logger, Model 21X, Campbell Scientific Inc., Logan, UT). Electronically recorded temperature data were taken every two hours and the average temperature over the two hour period recorded in the datalogger's internal memory. Electronic temperature data were downloaded from the datalogger through an RS-232 connection to an IBM-compatible computer using software provided by Campbell Scientific.

Moisture Content and pH of Bioreactors

The moisture content and pH of bioreactor matrices were performed as one procedure. The moisture content was first determined by placing triplicate 50 to 100 g wet bioreactor subsamples into Petri dishes after the bioreactor matrix had been homogenized. The Petri dishes with the matrix samples were then dried at 90 °C for approximately 48 hours. The percent H₂O content (H₂O weight/wet matrix weight, as well as H₂O weight/dry matrix weight) and wet matrix weight/dry matrix weight ratio...
were determined. Triplicate amounts of the wet matrix were weighed into 50 mL beakers based on the following equation: [0.8 x wet matrix weight/dry matrix weight]. Approximately the same volume of distilled H₂O (20 mL) was added to each beaker containing the wet matrix. The relationship, 0.8 x wet matrix weight/dry matrix weight, was predetermined in previous experiments. The intention was to add equivalent amounts of the matrix based on the dry weight. However, each individual matrix is hydrated at different levels and it is difficult to standardize the amount of matrix based on wet weight and its volume. It was decided that the matrix should be approximately 20 mL volume and distilled water added to result in a 1:1 (v/v) slurry for pH determination. The slurry was allowed to stir slowly for approximately one hour with a magnetic stirring bar and stirring plate. The pH was then determined by placing a pH 4 and pH 7 standardized combination Ag/AgCl₂ electrode (Fisher Scientific) in the slurry for 10 minutes.

**Analyses of Oxygen and Carbon Dioxide Concentration in Bioreactor Matrix Air**

The concentration of oxygen and carbon dioxide in air collected from the bioreactor matrix was separated and quantitated using gas chromatography with a thermal conductivity detector (TCD). Bioreactor air was collected by pushing a specially-designed aluminum probe (Berry et al., 1993) into the bioreactor at least 25 cm deep. Inside the aluminum probe was 1 μm diameter polyethylene tubing secured with silicone caulking. To collect bioreactor air, a 60 mL plastic syringe (B&D) was used to flush air from the tip of the probe in the bioreactor through a 23 mL anaerobic glass test tube (Bellco Glass, Inc, Vineland, NJ), which had a butyl rubber septum and two 21-gauge syringe needles placed through the septum; one needle was attached to the gas probe and the other attached to an 8 cm piece of tubing. After two syringe volumes had flushed through the test tube, the tubing connected to the two needles in the septum was clamped shut. The same procedure was also performed for collecting a background air sample, usually 0.25 m above the ground.

To separate and quantitate amounts of the oxygen, carbon dioxide, and potentially methane, one of two different gas chromatographs (GC) were used. One GC was a GOW-MAC (Ottawa, Canada) with a TCD and fitted with one of two different 1.8 m by 2.1 mm (inner diameter) copper columns. One column containing Haysep packing (Supelco, Inc., Belfonte, PA) was used for separating CO₂ and CH₄. The other column containing molecular sieve 5A (45-60 mesh, Sigma Chemical, Chicago, IL) was used for separating O₂. The carrier gas of helium was at a flow rate of approximately 60 mL/min
for both columns. The following instrument parameters were generally used: the bridge
current set at 140 mA, column temperature either 25 or 30 °C, detector temperature
was 185 °C, and the injector temperature was 50 °C.

The other GC was a Hewlett Packard Model 5890A equipped with a TCD and one
of two different 1.8 m by 2.1 mm (inner diameter) stainless steel columns. One column
was packed with Poropak N (80/100 mesh) for separating CO₂ and CH₄. The other
column packed with molecular sieve 5A (45-60 mesh, Sigma Chemical) was used for
separating O₂. Helium was used as the carrier gas at a flow rate of approximately 20
mL/minute. Detector, injector, and column temperature were set at 40 °C.

Gas standards of O₂ and CO₂ purchased from Supelco Inc. were injected (20 to
100 µL) into either GC-TCD using a gas-tight Hamilton syringe (50 or 100 µL) and the
GC responses recorded and printed by an attached integrator. The O₂ standard was 20% 
(w/w) and the CO₂ standard was 1% (w/w). Injections of sample gas were also made
using a gas-tight syringe and the molarity was determined by extrapolating from the GC-
TCD response of a comparable standard.

**Pesticide Residue Analyses**

There were several different extractions methods and chromatographic systems
employed in these experiments. The analytical methods used depended on the pesticide,
instrument availability, and when the experiment was performed. There was an evolution
of method development over time. The details of the pesticide residue analysis for each
experiment are outlined in each of the experiments' Sections.

**Statistical Analyses**

When possible and appropriate, results will be presented as a mean ± the standard
error of the mean (SEM).

Calculations of the dissipation time for 50% parent compound disappearance
(DT₅₀) were calculated using pseudo-first order rate kinetics and the concentrations of the
parent pesticide recovered from the solvent-extractable residue using HPLC. The
following equation represents the DT₅₀ calculation:

\[ DT_{50} = \ln \frac{2}{k} \]

where the DT₅₀ is the time it takes 50% of the original amount of pesticide to dissipate,
and the "k" is the DT₅₀ coefficient unique to each pesticide in each situation. The DT₅₀
coefficient \((k)\) is the slope of line from a plot of the ln of the pesticide concentrations or percentages vs. their respective times on the abscissa.

4.3.2.3 Experimental Design

Four distinct series of composting experiments were performed over approximately two years at the Virginia Tech Turf Grass Research Center. Additional supporting laboratory experiments were also performed.

4.3.2.3.1 Experiment 1: Atrazine and Chlorpyrifos Dissipation in Peat Moss Compost Contained in 189 L Rubbermaid® Containers

This first experiment involved 189 L plastic containers supplied by Rubbermaid® Co. (Twinsburg, OH). To prevent leakage, the 189 L containers were placed into larger plastic container liners. Twenty of the liners were sunken into the ground for insulating purposes and to help prevent them from being tipped over. An example of an 189 L bioreactor is illustrated in Figure 19.

The experiment was designed to monitor the dissipation of atrazine and chlorpyrifos in a composting matrix composed of peat moss using corn meal and nitrogen amendments. Approximately 10 kg of dry peat moss was added to two of the 189 L Rubbermaid® containers. Tap water was added to bring the hydration level to approximately 75% (water/wet matrix). Samples were taken for pH analysis and approximately 350 g of Ca(OH)\(_2\) was added to increase the peat moss pH. The compost matrix in these bioreactors was mixed well with a gasoline powered motor equipped with a specially designed auger. After approximately 1 month, samples were taken again for pH.

Atrazine formulated as AAtrex® 4L and chlorpyrifos formulated as Dursban® 4E was applied on 10 July 1990 at the intended rate of 10,000 and 20,000 μg/g dry matrix, respectively, to the two different 189 L Rubbermaid® containers. The pesticide application was done by spraying each formulated pesticide with a one gallon pesticide sprayer onto the peat moss matrix as it was being mixed by the gasoline-powered mixer.
Figure 19  Field Compost Structures
Additionally, 2.3 kg of corn meal was added to each of the atrazine and chlorpyrifos bioreactors, along with 10 g of urea dissolved in tap water, during the mixing process. The corn meal and nitrogen were reapplied and the compost was mixed approximately one and three months after the pesticides were initially applied.

Samples of the bioreactor matrix were taken for determining hydration levels, pH, and pesticide residue analysis before reapplying nutrients and mixing the bioreactor matrix. Approximately two liters of well-mixed bioreactor matrix from each container were put into four L glass jars. The contents were mixed for 6 hours on a roller-type mixer. Triplicate 10 g portions of the mixed matrices were removed and placed into 500 mL French square bottles along with 250 mL acetone. A Polytron ultrasonicator was used to extract the pesticides from the matrix, and the resulting supernatant was decanted and passed through Celite® 503 (J.T. Baker Chemical Co.) and Nuchar® C 190 N granular activated carbon (Fisher Scientific Co.) [2 +1 by mass] contained on a Whatman No. 5 cellulose filter in vacuo. The acetone extract was mixed with 250 mL aqueous Na₂SO₄ (5g/L) and partitioned into 100 mL of hexane. The partition was repeated two more times with 5 mL hexane. The combined hexane partition was mixed again with 200 mL of the aqueous Na₂SO₄ (5g/L), partitioned again, and the hexane phase passed through a column of Na₂SO₄. The hexane portion was taken to near dryness with the addition of 2 or 3 drops of Keeper's solution (2% paraffin oil in hexane), and then reconstituted with a known volume of hexane for chromatographic analysis.

The GC method used a Tracor 540 gas chromatograph equipped with an electron capture detector. A 1.8 m glass column (6.4 mm inner diameter) packed with OV-17/OV-210 (1.5%/1.95%) liquid phase on a 100/120 mesh Chromosorb W HP stationary phase (Supelco, Inc., Belfonte, PA). Instrument temperatures generally were: 235 °C (injector port); 185 - 205 °C (column); 350 °C (detector); and carrier gas flow rate, 4.0 mL/min N₂.

4.3.2.3.2 Experiment 2: Comparison of Biological Activity in Peat Moss-Based Compost and Steam-Exploded Wood-Based Compost in 189 L Rubbermaid® Containers with Different Treatments

There were two separate experiments performed to compare biological activity as influenced by bioreactor structure and nutrient amendments in the 189 L Rubbermaid®
Containers. Neither experiment involved pesticide dissipation as part of the experimental design.

One experiment involved the 189 L bioreactors containing either peat moss or steam-exploded wood. Treatments of corn meal, corn meal with a nitrogen amendment, or no additions were examined to observe the variations of biological activity as indicated by temperature. These experiments involved five different bioreactors. Three bioreactors contained approximately 15 kg dry steam-exploded wood (SEW) and the other two contained approximately 15 kg peat moss (PM). The matrix was hydrated with tap water and mixed well with a shovel. Corn meal (2.3 kg) and 5 g urea dissolved in tap water were added to a SEW and a PM bioreactor. To another set of bioreactors only corn meal was added. In one SEW bioreactor, no amendments were added. The contents of each bioreactor were again mixed well with a shovel. At various times during a month of incubation, air samples were taken from the bioreactors and analyzed for oxygen and carbon dioxide. Bioreactor temperatures and pH were also monitored.

The second experiment involved the same 189 L Rubbermaid® containers, but for comparative purposes, some were structured to allow good air flow through the bioreactor. Additionally, either corn meal or vegetable oil (waste oil from Burger King) was used as a nutrient amendment to compare biological activity as indicated by temperature between the two.

A plastic mesh (mesh: 2.5 cm wide by 1.9 cm high) basket (46 cm bottom diameter, 36 cm top diameter, 46 cm tall) was placed on the bottom of two 189 L containers with a 1 cm (diameter) polyvinyl chloride tube between the air above the bioreactor container and the void connecting the basket and the bottom of the container (Figure 19). One bioreactor was set up without the basket. The purpose of this basket structure was to permit good air flow through the bioreactor from the bottom. Approximately 12 kg (based on dry weight) of a wet mixture of peat moss and steam-exploded wood (1:1, v/v) was placed onto three different 3 m by 3 m plastic sheets on the ground. Corn meal (1.65 kg) and dried chicken manure (approx. 0.25 kg) were added to the matrix mix on two of the sheets of plastic. One liter of vegetable oil (deep fry oil waste from Burger King, Virginia Tech, Blacksburg, VA) was poured over the matrix mix on the third plastic sheet along with approximately 0.5 kg of dried chicken manure. The contents on each plastic sheet were mixed well with a shovel and placed into their appropriate bioreactors. Samples of bioreactor matrix air were taken at various times
during a two week period to determine oxygen and carbon dioxide concentration within the three bioreactors. Bioreactor temperatures were also monitored.

A separate laboratory experiment was performed to quantitate respired carbon dioxide from bioreactors composed of steam-exploded wood and separate treatments amending with corn meal + urea, corn meal only, or no nutrients. Two replicates of each of the treatments were made by adding steam-exploded wood based on 20 g dry weight to specially designed glass bioreactors (Figure 42 in Project VI). As a comparison, one adult male American cockroach (Periplaneta americana) was placed into one of the glass bioreactors. Corn meal was added to the appropriate bioreactors at a rate of 0.04 g/g dry matrix. Urea was added to appropriate bioreactors at a rate of 0.005 g/g dry matrix. The bioreactors were connected to the laboratory device designed by Petruska et al. (1985) (Figure 41 in Project VI) to collect carbon dioxide in a flow through system. The bioreactors were kept at 30 °C in insulated and temperature regulated 1 L battery jars. Once an hour for 2 minute periods, any respired carbon dioxide was trapped by passing air through the bioreactors and into burets containing 3 mL of a 3 M KOH solution. Every 24 hours for 11 days, the 3 mL of 3 M KOH solution was removed and replenished with fresh KOH solution. The collected KOH solution was diluted and a portion titrated with 1 M HCL to a phenolphthalein pH endpoint (pH = 8.3). The volume in milliliters of 1 M HCl used to achieve the phenolphthalein endpoint multiplied by the molarity of the HCl resulted in the milliequivalents (meq) of HCl used [meq HCl = volume HCl used x 1 M HCl]. The mmole of carbon dioxide trapped in the KOH solution was determined by dividing the milliequivalents (meq) of carbonate in the KOH solution by a dilution factor [mmole carbon dioxide = (dilution factor/2) x meq HCl used]. The dilution factor is the total diluted volume divided by the aliquot taken for titration. The meq amount of carbonate was determined by subtracting the meq amount of HCl used from the total alkalinity [total meq carbonate = total alkalinity - meq HCl used]. Total alkalinity is defined as the meq of KOH originally present in the buret corrected for the dilution and volume taken for the titration [total alkalinity = volume taken for titration x [(volume of KOH in buret x 3 M KOH)/ total diluted volume].
4.3.2.2.3 Experiment 3: Pesticide Dissipation in Compost in Ringer® Plastic Bag Containers and Compost Contained by Galvanized Steel Mesh Cylinder (one meter diameter)

One of the two composting bioreactor designs tested shown in Figure 19 was an approximately 120 L plastic bag manufactured by Ringer® (Eden Prairie, MN) specifically designed for composting. The bag had holes through the bag to allow air flow through the bioreactor matrix. The second bioreactor structure (Figure 19) was a cylinder (1.22 meters in diameter) made by wrapping 1 inch (2.54 cm) by 1 inch galvanized steel mesh that was 4 ft tall (1.22 meters) around five steel posts that were designed for the fencing. The dissipation of atrazine, chlorpyrifos, and malathion was studied over a 16 to 20 week period in each of the bioreactor schemes.

Small cloth (Rayon®) bags (10 cm by 10 cm) were made to contain compost matrix treated with pesticides. Bags containing pesticide-treated bioreactor matrix were placed within the actual compost, thus they were subjected to the same composting conditions as the entire compost. At specific times, replicate bags could be removed for pesticide residue analysis. The concept for these bags originated from Yu et al. (1991) and the Snell Environmental Group, Inc. (1982). The advantages to using the cloth bags were that similar replicates could be removed at various times and the experimental preparation did not require mixing large amounts of pesticide into the entire bioreactor.

The application of the pesticides was accomplished by making a biobased matrix mixture, followed by blending pesticide into portions of the bioreactor matrix mixture. The bioreactor mixture was made by combining ground peanut hulls, peat moss, steam-exploded wood, and steam-exploded peanut hulls in equal amounts (approx. 4 kg) in a 189 L container. The entire mixture was homogenized well and amounts equaling 50 g dry were placed into 750 mL mixing containers. Atrazine formulated as formulated as AAtrex® 4L, chlorpyrifos formulated as Dursban® 4E, and malathion formulated as Dragon® 50% E was mixed with water and added to each container with the 50 g dry mixture to result in a final concentration of approximately 1000 µg/g of each pesticide. The contents of each container were mixed well, and placed into the cloth bags which were then stapled shut.

The actual bioreactor consisted of peat moss, steam-exploded wood (SEW), steam-exploded peanut hulls (SEPH), peanut hulls, and dried oak and poplar leaves. Large amounts of each of the matrices were mixed together on the ground. The
approximate proportion by volume of the matrices used in the mixture in order of decreasing amounts were 50% leaves, 20% peat moss, 15% peanut hulls, 10% SEW, and 5% SEPH. Soil from the Turf Grass Research Center was added to the mixture, as well as four bags of the Brown Leaf Compost Maker® inoculum from Ringer® Corporation (Eden Pararie, MN). Corn meal (9 kg) and 20 L of dried chicken manure were added to the mixture. Tap water was added as the bioreactor matrix was mixed well. Three portions of approximately 57 L of the bioreactor matrix were added to three 20 L Ringer® composting bags. The remainder was used to construct the 1 m diameter cylindrical bioreactor.

The 1 meter diameter cylindrical bioreactor enclosure was constructed by first staking out five steel post outlining an approximate 1 m diameter circle. The 152 cm tall galvanized steel mesh (5 cm by 5 cm mesh) screen was wrapped around the five posts and secured to each one of the posts. Black polyurethane plastic sheeting was draped around the fencing. An additional portion of the galvanized steel mesh fencing was secured around the bioreactor structure.

Peanut hulls were placed on the floor of the cylinder to a depth of approximately 30 cm. On top of the peanut hulls was alternating layers of evenly spread leaves (19 L), 0.2 kg corn meal, 4 to 6 L of dried chicken manure, 4 to 6 L of the remaining bioreactor mixture left over after constructing the bagged composting bioreactor, and finally a layer (5 cm) of peanut hulls. Tap water was added as each entire sequence of bioreactor materials were put into the bioreactor structure. Four entire sequences of bioreactor materials was put into place. A final layer of peat moss covered the bioreactor. Temperature probes were placed in each of the composting bioreactor bags and in various places in the cylindrical bioreactor. Temperature data were recorded and stored electronically for future retrieval.

The cloth bags containing the pesticides were placed into the bioreactor units as close to the center as possible on 5 March 1992. Bags of different pesticides were placed into the cylindrical bioreactor away from one another to prevent cross-contamination. There were three different Ringer® compost bags, each containing a different set of pesticides in cloth bags. The matrix of the bagged bioreactor and the matrix of the cylindrical bioreactor was mixed approximately every 2 weeks on days 20, 37, 55, 69, 83, 97, 116, and 141 after experimental initiation. Nutrients consisting of approximately 1.1 kg corn meal and 2 to 3 L of dried chicken manure were added to the bagged bioreactor, and 2.3 kg corn meal and 10 to 12 L of dried chicken manure were added to the
cylindrical bioreactor on days 20 and 97 after experiment initiation. Vegetable oil (approx. 10 L) in the form of deep frying oil waste from Burger King (Virginia Tech, Blacksburg, VA) and 10 to 12 L of dried chicken manure were added to the cylindrical bioreactor on day 116 after experiment initiation.

Four replicate bags of each pesticide tested were taken from the bagged bioreactor the day the pesticides were applied to the bioreactor and on days 20, 37, 55, and 116 after experiment initiation. Three to four replicate bags of each pesticide were removed from the cylindrical bioreactor the first day the pesticides were applied to the bioreactor and on days 20, 37, 55, and 141 after experiment initiation. All samples were placed into airtight plastic storage bags and stored at -20 °C until extraction. All samples taken from the bagged and cylindrical bioreactor were thawed to room temperature and extracted at the same time.

The contents of each sample bag were mixed well by hand in 500 mL glass beakers and the weight of matrix recorded. The ratio of wet vs. dry matrix from each sample was determined by transferring approximately 10 g of the wet matrix into 1.0 g aluminum weighing dishes, recording the wet matrix weight, drying the matrix at 110 °C for 48 hours, and recording the dry matrix weight. Based on the wet/dry ratio, weighed portions of the wet matrix approximating 1.0 g dry matrix were transferred to 40 mL polypropylene centrifuge tubes (Fisher Scientific Inc., Atlanta, GA). Twenty mL of ethyl acetate was added to each centrifuge tube containing the matrix, the contents shaken by hand for one minute, and allowed to sit overnight, not exceeding 12 hours. The centrifuge tubes were then placed into a sonicating water bath (Fisher Scientific) for three separate 30 minute periods with 30 minute intervals of not sonicating. The contents were centrifuged at approximately 3000 rpm (1000 g; Beckman Centrifuge, Beckman Instrument, Inc., Irvine, CA) for 15 minutes. The supernatant was decanted into 500 mL beakers, which were place in a fume hood to reduce the volume. The extraction process was repeated for a total of four times, repeating the addition of 20 mL of ethyl acetate, the sonication process, the centrifugation, and decanting the supernatant into the same 500 mL beaker. After the fourth extraction the matrix was allowed to dry before being stored at -20 °C. The volume of the collective supernatant from each bioreactor was reduced to approximately 45 mL in a fume hood and then adjusted to 50 mL with ethyl acetate. Twenty mL aliquots were taken and stored at -20 °C and an additional four mL aliquot was taken for chromatographic analysis.
Previous recovery experiments involving spiking small amounts of peat moss (2 g dry) with 1 mg atrazine and chlorpyrifos standards were quite variable and dependent on the amount of time allowed before extraction. Average atrazine recoveries using the above extraction procedures were $72 \pm 9\%$ (mean $\pm$ sem), ranging from 52 to 87%. The 52% recovery resulted from samples allowed to sit at room temperature for 48 hours before extraction. Average chlorpyrifos recoveries were $94 \pm 3\%$ of originally applied, ranging between 92 to 107%. The 48 hour sitting time did not appear to affect the recovery as it did with atrazine. Recoveries involving malathion were not performed.

Atrazine and malathion were analyzed by gas chromatography. The gas chromatographic method employed a Tracer 540 gas chromatograph equipped with an electron capture detector. A 1.8 m glass column (6.4 mm inner diameter) was used with an OV-17/OV-210 (1.5%/1.95%) liquid phase on a 100/120 mesh Chromosorb W HP stationary phase (Supelco). Instrument temperatures generally were: injection port, 235 °C; column, 180 °C; detector, 350 °C; and carrier gas flow rate between 3 and 3.5 mL/min N$_2$. The retention times for atrazine and malathion were generally 4.3 and 12.2 minutes.

Chlorpyrifos was analyzed using high performance liquid chromatographic (HPLC) methods. The HPLC unit used was an Isco (Lincoln, NE) pump (Model 2350) and UV-VIS detector (Model V4). The column used for separating chlorpyrifos was an Isco Perisorb C-18 column (250 mm long by 4.5 mm inner diameter, 5 μm particle size). The average retention time for chlorpyrifos was 4 minutes.

Peaks in the chromatogram were identified by retention time (Rt) comparison to retention times of known standards. In certain circumstances, confirmation of peak identity was done by spiking the same sample with a standard and then repeating the chromatography. Quantitation of analyte amounts was done by extrapolating the UV response of the analyte to a standard curve of the compound being quantitated. Standards of atrazine and malathion prepared between 0.002 μg/g and 20 μg/g (ppm) were used to construct a standard curve using the gas chromatograph. A chlorpyrifos standard curve on the HPLC was constructed with injections of between 2 and 200 ng chlorpyrifos injected. A standard curve consisted of at least three different amounts of the standard and coefficients of determination ($r^2$) were greater than 0.94. Based on the extraction volumes, the volume injected onto the gas chromatograph, the standard amounts used, and the detector sensitivity, the limit of determination (LOD) for atrazine and malathion was determined to be 0.25 μg/g atrazine and 0.03 μg/g malathion. The LOD of chlorpyrifos in the HPLC system was 1 μg/g.
4.3.2.2.4 Experiment 4: Experiments Involving One Cubic Meter Composting Containers ('Birdhouse' Composting Container)

An enclosed one cubic meter composting bioreactor structure was built as shown in Figure 19. The intention behind constructing this compost system was to incorporate the larger composting size that was used with the cylindrical bioreactor, and also to determine the extent to which pesticide volatilization occurs in a situation where composting bioreactor temperatures exceed 50 °C. Four units were built in the same manner. The floor of the composting bioreactor units was constructed from 2 by 4 inch (5.08 by 10.16 cm) studs with galvanized steel mesh (0.635 cm) hardware cloth. Holes (1.905 cm) were drilled through all floor studs to allow good air flow. The walls of the composting bioreactor units were made of 1/2 inch (1.27 cm) thick plywood in a one cubic meter box, which was secured to the floor with metal "L" brackets. One of the walls was hinged on the side to act as a door. Clasps were secured to the other side to tightly close the door. Thin aluminum flashing covered the inside of the composting bioreactor walls, including the door. The top was constructed from 1/4 inch (0.635 cm) plywood with a wooden frame. The top was designed as a four sided pyramid and wide enough to hang over the edge of the composting bioreactor box structure so that rain would drain away from the unit. A vinyl hose gasket was secured to the top of the box portion of the unit to achieve a tight seal with the top when it was placed onto the box portion. There were clasps on each side of the top to tightly secure it to the composting bioreactor box. All seams in the composting bioreactor unit were filled with silicone caulking. Two holes (2.5 cm) were drilled in the apex of pyramidal top and fitted with two 1.5 inch (3.8 cm) polyvinyl chloride (PVC) tubes with a 90° elbow.

Pesticides were applied to a mixture of compost matrices (4.5 kg) and then the pesticide + matrices were placed into 61 cm tall by 20 cm diameter cylinders (approx. 19 L) made from galvanized steel mesh (1/4 inch or 0.635 cm) hardware cloth. The initial volume of the matrices in the cylinder was approximately 15 L. The cylinders were then placed in the composting bioreactor structures in a similar experiment design used in the bag and cylindrical bioreactors (Section 4.3.2.2.4). The mixture of composting matrices was composed of equal amounts of peat moss, steam-exploded wood (SEW), steam-exploded peanut hulls, shredded peanut hulls, and shredded leaves (oak and poplar). The shredding was done using a mechanical yard shredder. Portions (4.5 kg) of the dry matrix were placed into four different 38 L plastic containers. Atrazine formulated as AAtrex®
4L, chlorpyrifos formulated as Dursban® 4E, and malathion formulated as Dragon® 50% E were added to containers containing the 4.5 kg dry compost matrix mixture. One 38 L container contained only the matrix mixture without the pesticide and served as a blank. Corn meal (0.23 kg) and 1 L of dried chicken manure were also placed into each container. Tap water was added as the contents in each container were mixed well by hand. The amount of composting matrix was weighed and samples were taken for hydration levels, pH, and initial pesticide residue analysis. The remainder in each of the containers was placed into the galvanized steel mesh cylinders, which were put into the middle of the composting bioreactor structure. Each of the four composting bioreactor units were designated for each of the pesticides tested (1) no pesticide blank, (2) malathion, (3) chlorpyrifos, and (4) atrazine.

The larger composting bioreactor was made of a mixture of approximately 50% fresh grass clippings, 20% shredded leaves, 20% peanut hulls, and 10% steam-exploded wood (SEW). Approximately 30 cm of peanut hulls were placed on the composting bioreactor floor. The cylinders containing the pesticide-laden matrix were put on top of the peanut hull layer in the middle of the composting bioreactor unit. Layers of grass clippings, chicken manure (4 L), peanut hulls, peat moss, and SEW were placed sequentially on top of each other around the pesticide cylinders. Layers were continually added until the composting matrix was approximately 1 m tall. Temperature probes were placed into the composting matrix and temperatures were recorded electronically every 2 hours for later retrieval.

Pesticides were applied to the matrix in the cylinders, the cylinders were put into the composting bioreactor units, and the composting bioreactors were constructed on 24 July 1992. The matrix in each composting bioreactor unit was pulled out of the composting bioreactor structure and mixed on the ground on days 7, 37, 54, 72, 93, 121, 161, and 228 after experiment initiation. Additional grass clippings and chicken manure were added to each composting bioreactor on day 54. The pesticide cylinders were removed from the composting bioreactor on days 7, 37, 72, and 252 after experiment initiation. The contents were mixed well by hand, and four approximately 250 mL portions were put into plastic storage bags for hydration levels, pH, and pesticide residue analysis. All samples were placed into airtight plastic storage bags and stored at -20 °C until extraction. Samples taken from the cubic meter bioreactor experiments were thawed to room temperature and extracted at the same time.
The contents of each sample bag were mixed well by hand in 500 mL glass beakers and the weight of matrix recorded. The ratio of wet vs. dry matrix from each sample was determined by transferring approximately 10 g of the wet matrix into 1.0g aluminum weighing dishes, recording the wet matrix weight, drying the matrix at 110 °C for 48 hours, and recording the dry matrix weight. Based on the wet/dry ratio, weighed portions of the wet matrix approximating 1.0 g dry matrix were transferred to 40 mL polypropylene centrifuge tubes (Fisher Scientific). Twenty mL of ethyl acetate was added to each centrifuge tube containing the matrix, the contents shaken by hand for one minute, and allowed to sit overnight, not exceeding 12 hours. The centrifuge tubes were then placed into a sonicating water bath (Fisher Scientific) for three separate 30 minute periods with 30 minute intervals of not sonicating. The contents were centrifuged at approximately 3000 rpm (1000 x g; Beckman Centrifuge) for 15 minutes. The supernatant was decanted into 500 mL beakers, which were place in a fume hood to reduce the volume. The extraction process was repeated for a total of four times, repeating the addition of 20 mL of ethyl acetate, the sonication process, the centrifugation, and decanting the supernatant into the same 500 mL beaker. After the fourth extraction the matrix was allowed to dry before being stored at -20 °C. The volume of the collective supernatant from each bioreactor was reduced to approximately 45 mL in a fume hood and then adjusted to 50 mL with ethyl acetate. Twenty mL aliquots were taken and stored at -20 °C and an additional 4 mL aliquot was taken for chromatographic analysis.

Atrazine and chlorpyrifos were analyzed by HPLC. Malathion was analyzed using the gas chromatographic method described in Section 4.3.2.2.3. The HPLC method for atrazine and chlorpyrifos was performed using a Hewlett Packard HPLC unit (Model No. 1050; Hewlett Packard Co., Avondale, PA) with a low pressure solvent-mixing unit, a Rheodyne (Coati, CA) Model 7125 injection valve fitted with a stainless-steel 20 μL injection loop, and a ultraviolet-visible (UV-VIS) detector (Hewlet-Packard Model No. 1050). The chromatographic column used for all analyses was a Nucleosil® C18-HS-bonded silica column (250 mm long by 4.6 mm in internal diameter, 5 μm particle-size, 60 Å pore-size, 18% w/w carbon load, endcapped silanols; Alltech). All solvents were continuously sparged with helium, and flow rates were maintained at 1.0 mL/minute.

The chromatographic analysis of the atrazine samples was based on the method described by Wenheng et al. (1991) and performed under reverse-phase conditions using an ion-pairing water/acetonitrile ternary gradient at a fixed wavelength of 225 nm. The solvent reservoirs consisted of a H2O, acetonitrile (ACN), and a 50/50 H2O/ACN
reservoir. The H₂O and 50/50 H₂O/ACN reservoirs were 0.020 M n-heptanesulfonic acid (Kodak, Hartford, CN) and 0.10 M H₃PO₄. Table 15 represents the gradient:

Table 15  HPLC Gradient for Atrazine and Several of its Metabolites

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>H₂O Reservoir</th>
<th>ACN¹ Reservoir</th>
<th>50/50 H₂O/ACN Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>37</td>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

¹ACN is acetonitrile

The retention times in minutes of atrazine and metabolite standards were 13.4 minutes for hydroxyatrazine and 21.7 minutes for atrazine. Based on the sensitivity of the UV-VIS detector for standards of atrazine at a wavelength of 225 nm and the volume of the extract, the limit of determination (LOD) for atrazine was 0.5 µg/g dry matrix (ppm).

The chromatographic analysis of the chlorpyrifos samples using a modified method described by Racke et al. (1990), which was performed under reverse-phase conditions using a water/methanol binary gradient at a fixed wavelength of 300 nm. Both solvent reservoirs were 0.05% (w/w) N,N-diethyl-octylamine (Sigma Chemical, Chicago, IL) and 1% (v/v) acetic acid. The gradient used was as follows in Table 16:

Table 16  HPLC Gradient for Chlorpyrifos and 3,5,6-trichloropyridinol

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Percent of Total Flow (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H₂O Reservoir</td>
</tr>
<tr>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

¹ACN is acetonitrile

The retention times for standards of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) were approximately 11 and 6 minutes, respectively. Based on the sensitivity of the
UV-VIS detector for chlorpyriphos and TCP at a wavelength of 300 nm and the volume of the extract, the LOD for chlorpyriphos and TCP in this study was 5 μg/g dry matrix (ppm).

4.3.4 Results

4.3.4.2 Experiment 1: Atrazine and Chlorpyriphos Dissipation in Peat Moss Compost Contained in 189 L Rubbermaid® Containers

Figure 20 represents the compost temperatures over the approximate 4 month period of incubation. The peak in temperatures was in direct correlation with the initial amendment with corn meal + urea and additional similar amendments after 31 and 87 days after experiment initiation. Peak compost temperatures between 35 and 40 °C appeared to be slightly greater in the chlorpyriphos bioreactor compared to the atrazine bioreactors. Peak temperatures appeared to last approximately 2 weeks. Compost temperatures remained near ambient temperature until further nutrient amendment occurred. Increased temperatures in these 189 L bioreactors occurred because of the nutrient amendment, therefore, more frequent nutrient amendments would need to be done to maintain increased temperatures.

The atrazine concentration was initially determined to be 8947 μg/g, and decreased to 5277 and 5200 μg/g after 31 and 87 days of incubation, respectively. The DT₅₀ of atrazine in this compost situation was determined to be 99 days (r² of 0.979). The chlorpyriphos at experiment initiation was determined to be approximately 22,000 μg/g. The chlorpyriphos concentration decreased to 16,060 and 14,410 μg/g after 31 and 87 days, respectively. The DT₅₀ for chlorpyriphos in this composting situation was determined by first order rate kinetics to be approximately 136 days (r² = 0.994).

The hydration of the atrazine compost was slightly higher at approximately 81% (water/wet matrix; w/w) than the chlorpyriphos compost at 76% hydration. General recommendations of compost hydration were between 50 and 70%, and levels greater than that decrease air flow and anaerobic conditions can prevail (Golueke, 1972; Polprasert, 1989). However, Mathur (1991) reports that in his research with peat moss based composts, the hydration levels generally are between 70 and 90% water/wet.
Figure 20  Field Compost Trials in 189 L Rubbermaid BioReactors
matrix; w/w. Peat moss on weight per weight basis is a better absorbent material that equal weights of common compost materials, such as grass clipping, straw, and leaves. Peat moss can hold up to 20 times its dry weight in water (Bulganina et al., 1983). The goal of optimal composting is to have enough water for biological activity without decreasing airflow and the oxygen content of the compost.

The pH of the peat moss based compost before atrazine and chlorpyrifos were applied was approximately 3.2. To increase the pH of the peat moss, Ca(OH)\textsubscript{2} was added approximately 1 month before the initiation of the experiment. After 1 month the pH was 6.2 at the time of the initiation of the experiment. The pH of sphagnum peat moss is generally between 3 and 4 (Bulganina et al., 1983), and Ca(OH)\textsubscript{2} was added to the compost 1 month prior to experiment initiation for the purpose of increasing the pH. It was believed that increasing the pH to neutral conditions would be a better situation for microorganisms inhabiting the compost (Mathur, 1991). The pH of the atrazine compost was 6.1 at experiment initiation. The atrazine compost pH increased to 6.6 after 31 days, and after 87 days the pH was 5.4. Similarly, the pH decreased slightly during the approximate 3 month incubation period, pH of 5.6 and 5.4 after 31 and 87 days, respectively.

In some composting situations, there is an initial increase in pH as ammonia is formed from the protein degradation, but the pH usually decreases back to near neutral conditions. Polprasert (1989) states that as long as the composting process remains aerobic, the pH should not change significantly. The observed pH decline may be more a result of the buffering capacity of the peat moss rather than any process attributed to the microorganisms present in the peat moss based compost. A decline in pH may not be beneficial for microorganisms, but acidic conditions should improve the hydrolysis of atrazine and chlorpyrifos. Both atrazine and chlorpyrifos are more susceptible to hydrolysis in conditions of decreasing pH (Skipper et al., 1967; Macalady and Wolf, 1983).

The 189 L bioreactors used in this composting situation to detoxify atrazine and chlorpyrifos at high initial concentrations of approximately 9000 and 22,000 µg/g, respectively, were effective in reducing the pesticides by nearly 40% after 3 months of incubation. It was concluded that even though the pesticide dissipation was good, measures should be taken to ensure quicker rates of pesticide dissipation. Microbial activity, their role in degrading pesticides, and improving microbial activity became a focus of the research.
4.3.2.3.2 Experiment 2: Comparison of Biological Activity in Peat Moss-Based Compost and Steam-Exploded Wood-Based Compost in 189 L Rubbermaid® Containers with Different Treatments

A set of experiments using the 189 L Rubbermaid® bioreactors containing either peat moss (PM) or steam-exploded wood (SEW) were performed to compare temperatures and oxygen and carbon dioxide concentrations with different treatments. One treatment involving bioreactors containing either PM or SEW had amendments of either corn meal + urea (or chicken manure) or no amendment. Another treatment involved adding a basket structure to the bottom of the 189 L bioreactor to improve air flow (Figure 19). In addition to the field studies, a separate laboratory experiment was performed to compare the amount of carbon dioxide respired from compost amended with and without nutrients were compared.

From Figure 21, it is obvious that adding corn meal + urea increased compost temperatures. In the nutrient amended compost bioreactors containing either PM or SEW, temperatures rose above 35 °C, but remained below 25 °C in compost not amended with nutrients. Even though temperatures were greater in compost with nutrients added, carbon dioxide concentrations were fairly similar between treatments of nutrients or no nutrients in both the PM and SEW composts (Figure 21). It is difficult to conclude based on the carbon dioxide data presented in Figure 21 that overall metabolism was similar between the treatments. The difficulty exists because compost carbon dioxide was collected as interstitial compost air and quantitated as a concentration, not as the total mass of carbon dioxide respired.

Increased carbon dioxide concentration indicates that respiration is occurring, and if the airflow through the compost is passive, it may be proportional to the degree of respiration. It is interesting to note that the oxygen concentration in the compost pits was inversely proportional to the carbon dioxide concentration. Oxygen concentrations were lowest when carbon dioxide concentrations were greatest. As the carbon dioxide concentration decreased towards the end of the 26 day incubation period in all treatments, oxygen concentrations then increased back near normal background oxygen concentrations of 8.6 x 10⁻³ M (20% oxygen). However, this was not necessarily the case in one SEW bioreactor with no nutrient amendment (Replicate 1) (Figure 21), which had a comparatively lower oxygen concentration at the end of the incubation period.
Figure 21  Pit Temperature, Carbon Dioxide Respiration, and Oxygen Content in Different Matrices and Various Nutrition Regimes
compared to other bio-reactors. It may have been that the matrix was more hydrated in that particular compost, because if air could not flow readily due to too much water, anaerobic conditions would have occurred. Methane was not observed in any of these pits, therefore, the low oxygen levels observed in the one SEW replicate were not low enough to support methanogenic bacteria. Methanogenic bacteria cannot tolerate oxygen, but other micro-aerophiles and facultative anaerobes can tolerate small amounts of oxygen (Paul and Clark, 1989). Detection of the facultative anaerobes and micro-aerophiles would have been done by microbiological means other than detection of respired gases, since they respire carbon dioxide.

There is some variability in the reported recommendations for minimum oxygen content in compost that supports optimal biological activity. It is important to maintain approximately 10% oxygen in the interstitial air space within the compost for optimum aerobic microbial activity (Mathur, 1991). Evidently, the thermophilic composting conditions require between 5 and 7% oxygen. Oxygen percentages much lower tend to limit the biological activity, which may reduce the rate at which organic degrade through biological means.

It is important to have adequate carbon and nitrogen available for microbial growth in a composting environment (Biddlestone et al., 1987). Assimilating nitrogen is essential for cellular growth. The presence of an easily metabolized carbon source does not guarantee good microbial growth and activity unless the microbial population has access to an adequate nitrogen source. The carbon to nitrogen ratio (C:N) of matrices used to construct a compost is important to consider. In general, it is important to have adequate nitrogen in compost, and matrices with low C:N ratios on a weight to weight basis have more nitrogen than matrices with higher C:N ratios. The importance of nitrogen in compost conferring optimal microbial activity does not preclude the importance of ensuring that there is a good carbon source in the compost. Peat moss and steam-exploded wood are commonly used in the pesticide waste disposal system being developed at Virginia Tech because of their sorbative qualities in the primary isolation phase of the disposal system (Figure 1: Chapter 1, Introduction). However, they do not necessarily provide easily metabolized carbon nor have good C:N ratios, and that is why nutrient amendments of corn meal and urea for nitrogen have been a normal management practice in composting pesticides at Virginia Tech.

A laboratory experiment was performed to determine what effect amending steam-exploded wood compost with either corn meal or corn meal + urea had on microbial
activity. It was obvious in past experiments that by adding corn meal to compost, compost temperatures increased. The steam-exploded wood (SEW) being used as a sorbent in the isolation phase of the pesticide waste disposal system would eventually be composted. It was believed that the SEW could act as a good energy source for microbial growth due to its larger surface area which is exposed during the steam-explosion process.

It is apparent from Figure 22 that the greatest microbial activity indicated as the total respired carbon dioxide was in the bioreactors amended with corn meal and urea. This experiment stressed the importance of adding nitrogen to the peat moss and steam-exploded wood compost used in the compost designed for pesticide detoxification. It is interesting that the microbial activity of the control (no nutrients) was similar to the microbial activity observed in bioreactors amended with just corn meal (no urea). Concluded from this experiment is the need to have nitrogen present in a steam-exploded wood based compost. Also, it appears from Figure 22 that steam-exploded wood can support microbial activity without having corn meal present. However, previous field research showed that compost temperatures increased when corn meal was added.

Based on the design of the 189 L bioreactors, the nature of the peat moss or steam-exploded wood, and the fact that the only air flow into the bioreactors was through the top surface of the matrix, it was concluded that measures should be taken to improve aeration. An experiment was performed involving the 189 L bioreactors designed with a basket in the bottom to improve passive air flow through the compost (Figure 19). Also studied was the effect that adding vegetable oil in place of corn meal would have on microbial activity. It was believed that vegetable oil would provide an easily degradable source of energy and have a better spatial coverage over the compost matrices than corn meal.

The temperature data presented in Figure 23 shows that the highest compost temperatures were achieved in the bioreactor with a basket and with vegetable oil added as an energy source. There was an unfortunate loss of temperature data between circadian days 152 and 156 due to equipment failure, which explains the gap of data in Figure 23. The highest temperatures reached in the bioreactor amended with vegetable oil were close to 45 °C and an average between 35 and 40 °C over the month study. The temperatures of the bioreactor with a basket and amended with corn meal appeared to be slightly higher than the temperatures in the bioreactor without a basket, but amended with corn meal.
Figure 22 Carbon Dioxide Release from Steam-Exploded Wood Bioreactors Treated with Corn Meal, Corn Meal + Nitrogen, or No Amendment

0.8 g Corn meal was added to 20 g steam-exploded wood (SEW) (dry weight basis)
For the nitrogen amendment, 0.1 g urea was added to 20 g SEW (dry weight basis)
Carbon dioxide release from one male adult P. americana or American cockroach was compared
The presence of the basket may have resulted in giving only slightly higher temperatures compared to not having the basket. It was interesting to find that oxygen concentrations were similar between all three bioreactors studied with and without the basket (Figure 24). It was originally thought that the air flow from the bottom of the compost would help flush carbon dioxide from the compost air space and keep oxygen concentrations higher than the 7 to 10% minimum recommend for optimal aerobic composting (Mathur, 1991). In all three bioreactors oxygen concentration decreased to 4% or less of the compost air until about midway through the 1 month incubation. After that the oxygen increased to approximately 10% of the compost air. Conversely and inversely proportional to the temporal oxygen concentrations were the carbon dioxide concentrations (Figure 24). As compost oxygen concentrations were low during the first half of the 1 month incubation, carbon dioxide concentrations increased to concentrations between \(8 \times 10^{-3}\) and \(1 \times 10^{-2}\) M compared to a normal background carbon dioxide concentration of \(4.2 \times 10^{-4}\) M. A depletion of readily metabolized carbon may be the reason for the slow decline observed with the carbon dioxide after about 2 weeks. A reason why the oxygen concentration was initially low and then slowly increased could be related to an increase in interstitial air space in the compost matrices. The increased air space may have occurred due to loss of water in the air space, or degradation of compost mass.

The combination of steam-exploded wood, vegetable oil, and the basket appeared to yield higher compost temperatures (Figure 23) than observed in peat moss bioreactors without a basket and amended with corn meal (Figure 20). It was believed that achieving temperatures between 45 and 50 °C in these 189 L bioreactors using SEW and PM is possible to maintain over prolonged periods of time if amendments of nitrogen and vegetable oil are added to the compost. It was concluded that in future research, bulking agents, such as wood chips, should be added to increase the oxygen concentrations to levels near 10% in the SEW and PM compost. Compost temperatures should increase above the 45 and 50 °C range if oxygen concentrations are increased.
Figure 24  Oxygen\textsuperscript{1} and Carbon Dioxide\textsuperscript{2} Concentration in Compost Contained in Baskets to Increase Aeration and in Compost Without Baskets

\textsuperscript{1}Background oxygen concentration was $8.6 \times 10^{-3}$ M

\textsuperscript{2}Background carbon dioxide concentration was $4.2 \times 10^{-4}$ M
4.3.2.3 Experiment 3: Pesticide Dissipation in Compost in Ringer® Plastic Bag Containers and Compost Contained by Galvanized Steel Mesh Cylinder (one meter diameter)

An experiment involving the fate of pesticides in two different compost designs were performed. One compost structure examined used 120 L plastic bags designed for composting and contained aeration holes. The second compost structure was an approximately 1 m diameter cylinder made from galvanized steel mesh fencing. In addition, peanut hulls were added for bulking to the composting matrices of PM, SEW, and dried leaves. It was believed that the peanut hulls would create good air spaces in the compost to promote good aeration. The cylindrical compost is one of the most common composting structures recommended (Golueke, 1972; Martin and Geshuny, 1992). Its use is composed of several optimized parameters for composting situations. A minimal 1 m by 1 m wide and 1 m high compost mass is recommended to achieve thermophilic conditions in compost with passive aeration (Golueke, 1972). The primary reason for the larger mass is insulation. In smaller masses, heat is easily lost, and in compost masses much larger than the 1 m³, forced air through the compost is required to keep conditions aerobic.

The temperature data from the bagged and cylindrical composts are presented in Figure 25. Due to an equipment failure early in the experiment, the temperature data presented in Figure 25 starts on circadian day 85, which was 20 days after the initiation of the experiment.

The temperature profile presented for the bagged compost is from one replicate and is a good representation of the temperature profiles from the other three compost bags. There was a temperature spike reaching 54 °C in the bagged compost on approximately circadian day 91, which was higher than temperatures observed in the 189 L Rubbermaid® bioreactors with and without a basket. The temperatures in the bagged compost did decrease after the initial heightened temperature peak, to temperature peaks near 35 °C after mixing episodes. The volume of the bagged compost was slightly larger than the compost in the 189 L bioreactors. The 189 L bioreactors were sunken into the ground for insulation, but the bagged compost was entirely exposed to the atmosphere. From Figures 23 and 25, it is not obvious whether or not the bagged compost may lose heat more readily than the 189 L bioreactors. Both compost structures do appear to have been affected by ambient temperatures once the initial temperature peak subsided.
The larger mass size in the cylindrical compost had a dramatic effect on internal compost temperatures, which reached 67 °C in the temperature peak observed on approximately circadian day 91 after corn meal and chicken manure were added on circadian day 85 (Figure 25). A second temperature peak was observed at approximately circadian day 106 after the compost was mixed on circadian day 103. The second temperature peak was a result of redistributing unmetabolized corn meal, chicken manure, and vegetative material. Further mixing episodes (days 120, 134, and 148) without adding nutrients did cause an increase in compost temperature, but the increases were only slight (approximately 5 °C).

Nitrogen appears to be as much of a limiting factor in good compost microbial activity as a readily metabolized energy source. On circadian day 162 an amendment of only chicken manure was added to both the bagged and the cylindrical compost, which resulted in a temperature spike of approximately 65 °C in the cylindrical compost a couple of days later. The nitrogen contents of SEW, PM, and oak leaves are fairly low, therefore, it is essential that materials used as a source of nitrogen are added to this high C:N ratio type of composting system. In fact, it may be necessary in the future to amend with nitrogen every one or 2 weeks as indicated from the gaps between temperature peaks in Figure 25.

Chicken manure and vegetable oil were added in lieu of corn meal, and they constituted the last amendment to the bagged and cylindrical composts on circadian day 181. The vegetable oil was deep frying oil waste from a Burger King fast food restaurant that was being stored for disposal. The compost temperature increased to near 40 and 65 °C in the bagged and cylindrical composts, respectively. The rise in temperature was expected with an amendment of chicken manure and vegetable oil, however, the unexpected result was that the compost temperatures recorded in both the bagged and cylindrical composts remained high for a longer period than in similar treatments with corn meal or just chicken manure (Figure 25). Higher compost temperatures were observed in a 189 L bioreactor (with basket) that had an amendment of vegetable oil (Figure 23). The higher and prolonged temperatures observed in composts amended with vegetable oil are most likely due to the greater coverage of the liquid vegetable oil and that lipids yield approximately twice the metabolic energy per weight as similar amounts of carbohydrates (Biddlestone et al., 1987). The growth of microbial populations are also limited by the space in which they can grow. Larger populations of microbes can be supported with the larger coverage of vegetable oil in the compost matrices.
There appeared to be a good correlation between compost temperature and pesticide dissipation. Figure 26 illustrates the pesticide dissipation observed in the bagged and 1 m cylindrical compost units. Malathion quickly dissipated in the bagged compost from an initial concentration of approximately 1000 μg/g to less than 1 μg/g after 8 weeks. The dissipation of atrazine was not as quick as the malathion, but after 16 weeks, approximately 33 μg/g or 1% of the original amount of atrazine (2425 μg/g) was left in the bagged compost. After 20 weeks in the cylindrical compost, the atrazine concentration was approximately 5 μg/g or approximately 0.2% of the original 2425 μg/g atrazine applied to the compost bags. The DT₅₀ values of atrazine in the bagged and cylindrical compost units were 17 days ($r^2 = 0.99$) and 14 days ($r^2 = 0.996$), respectively. The smaller and cooler bagged compost unit appeared to be as effective in detoxifying atrazine compared to the larger and hotter cylindrical compost unit.

However, the cylindrical compost unit appeared to be more effective in detoxifying chlorpyrifos than the bagged compost unit (Figure 26). The DT₅₀ values of chlorpyrifos in the bagged and cylindrical compost units were 50 days ($r^2 = 0.993$) and 20 days ($r^2 = 0.951$), respectively. The greater dissipation rate of chlorpyrifos in the cylindrical compost unit may have been associated with increased microbial activity. Even though it is believed by most researchers that chlorpyrifos degrades by primarily abiotic hydrolysis to 3,5,6-trichloro-2-pyridinol (TCP), there are reports that the hydrolysis is enhanced by microbial activity (Getzin and Rosefield, 1968; Miles et al., 1983). The presence of the hydrolytic product TCP has been shown to be toxic to soil microorganisms (Racke et al., 1988; Somasundaram et al., 1990), therefore, a system of heightened microbial activity might be affected less by the TCP compared to a system with less microbial activity.

Greater volatilization of chlorpyrifos in the elevated temperature cylindrical compost may have been another reason chlorpyrifos dissipated at a quicker rate than in the cooler bagged compost. Unlike atrazine which is not considered to be a volatile pesticide (vapor pressure = 0.037 mPa; 20 °C), chlorpyrifos is somewhat volatile with a vapor pressure of 2.53 mPa; 25 °C) (Howard, 1991). A greater amount of chlorpyrifos will volatilize with increased temperatures. Unfortunately, the degree of chlorpyrifos volatilization, as well as atrazine and malathion volatilization, was not determined in this particular study. Only the dissipation of the pesticides was quantified as the residue recovered from the cloth bags placed within the bagged and cylindrical compost.
Figure 26  Pesticide Dissipation in Bagged and 1 meter (d.a.) Cylindrical Compost

Vertical bars are standard errors of the means
The compost was open to the atmosphere, and it is difficult to conclude to what extent the pesticides, especially chlorpyrifos, volatilized from the compost.

It may be interesting to determine how pesticides dissipate in compost, but the important point to consider is the rates of pesticide dissipation in compost designed to detoxify waste pesticides. Apparently, a larger volume of compost does promote higher temperatures. Higher compost temperatures confer greater microbial activity and increase overall physicochemical reactions, which increases the rate of pesticide dissipation. It would seem of paramount importance to use larger compost structures with good air flow for detoxifying pesticides in a pesticide waste disposal system. Also learned from these experiments is the importance of keeping nitrogen plentiful in compost to maintain microbial growth and the desirable high temperatures. Dried chicken manure was free in this case and proved to be a concentrated source of nitrogen for promoting microbial growth and heightened compost temperatures. Additionally, using vegetable oil as an energy amendment was successful in promoting high compost temperatures which remained higher for a longer time than if corn meal were used as the energy supplement. The vegetable oil used was a waste product from a fast food restaurant. Supplemental sources of energy and nitrogen are needed, and it seems logical that waste products from human activity can be used effectively in this pesticide waste disposal system. It reduces the cost of the pesticide waste disposal system and results in recycled waste.

The question of how much of the pesticides being composted dissipated due to volatilization prompted another experiment that attempted to quantify volatilization. The experiment used a semi-closed composting unit to collect volatile organic compounds vented from the compost.

4.3.2.2.4 Experiment 4: Experiments Involving One Cubic Meter Composting Containers ('Birdhouse' Composting Container)

Enclosed one cubic meter compost units (Figure 19) were constructed to study pesticide dissipation, incorporating all of the compost improvements that were determined upon in the previous experiments for improving microbial activity. Because the compost structures were enclosed units, the compost exhaust air could be collected and analyzed for volatile pesticides. Pesticides were applied to the compost matrix and kept in small containers placed in the inside of the compost similar to the cloth bags used in the
previous experiment. The floor of the compost units were raised above the ground and covered with a layer of peanut hulls to promote good air flow through the compost matrix and out the vents in the top of the compost unit (Figure 19). As in the previous experiment, peanut hulls were added as a bulking agent to promote good air flow through the compost. In addition, fresh grass clippings were included as part of the compost matrix. They constitute a good source of carbon and nitrogen with a C:N ration of 12 to 15, which rivals chicken manure for the amount of nitrogen (Polprasert, 1989). The compost units were tightly enclosed except for the mesh bottom, where air would enter the compost, and the two top ports, which had plugs of polyurethane foam placed in them to trap volatile organic compounds including any volatile pesticide.

Compost temperatures in the four compost units were similar throughout the 36 week incubation and one representative temperature profile is illustrated in Figure 27. The experiment was initiated on circadian day 205 and the compost temperature increased quickly to $67^\circ$C. The compost material was redistributed by mixing or turning the compost after 1 week, which resulted in a temperature rise to $68^\circ$C (Figure 27). The temperature then decreased over the next month to about $20^\circ$C at which time another mixing episode was performed. There was a small temperature increase to approximately $40^\circ$C due to redistribution of the compost material. Fresh grass clippings were added on circadian day 252, and were mixed into the compost matrix a week later. A temperature peak near $60^\circ$C was the result of the new influx of energy and nitrogen to compost. Colder ambient weather began and only mixing episodes occurred until an amendment of grape must was added to the compost on circadian day 87, which resulted in a temperature increase to about $25^\circ$C near the end of the experiment (grape residue after pressing for wine making; source was the Department of Horticulture at Virginia Tech). The mixing episodes that were done during the colder months did not appear to increase internal compost temperatures to much of an extent.

The enclosed one cubic meter composts tested had a similar temperatures as observed in the cylindrical compost unit. Also, similar was the effectiveness of the compost units to detoxify atrazine, chlorpyrifos, and malathion in the compost. Malathion quickly dissipated from the compost from an initial concentration of approximately 2700 µg/g to 0.6 after 5 weeks of incubation. The DT$_{50}$ of malathion in this system was 3 days ($r^2 = 0.874$) compared to 5 days in the cylindrical compost. The dissipation of atrazine was somewhat quicker in the cubic meter compost with a DT$_{50}$ of
Figure 27. Field compost trials in one cubic meter birdhouse-shaped reactors.
2 days (r² = 0.981) compared to 14 days in the cylindrical compost. Atrazine in the cubic meter compost dissipated to approximately 4 µg/g after 10 weeks of incubation from an initial atrazine concentration of 3487 µg/g (Figure 27). Chlorpyrifos was added to cubic meter compost at a higher concentration of 7421 µg/g which declined to 2290 µg/g after 10 weeks of incubation. The DT₅₀ of chlorpyrifos in this compost system was 59 days (r² = 0.993).

Atrazine's major metabolite hydroxyatrazine (HYA) was quantitated during the 36 week incubation. The HYA concentration of approximately 167 µg/g remained fairly unchanged from the second week throughout the entire 36 week period (Figure 28). In fact the HYA concentration actually was slightly higher at 182 µg/g at the 36 week period. The major metabolite of chlorpyrifos 3,5,6-trichloro-2-pyridinol (TCP), which is a product of hydrolysis was present in the compost following a similar pattern as the HYA. Concentrations of TCP increased to approximately 646 µg/g at 5 weeks and remained at that approximate concentration for the duration of the experiment. Both HYA and TCP appeared to reach some equilibrium of being formed as a result of the parent pesticide hydrolysis, and being further degraded themselves. After 10 weeks of incubation, the atrazine and chlorpyrifos dissipation also remained unchanged for the remainder of the experiment. Of course, between 10 and 36 weeks of the experiment, compost temperatures decreased and followed the cold ambient temperatures of the winter months. It is likely that atrazine and chlorpyrifos dissipation would have increased if the compost temperatures had been higher (during warmer months) after the initial peaks, or the experiment was prolonged with increased compost temperatures.

Dissipation of atrazine, chlorpyrifos, and malathion can occur by abiotic mechanisms, as well as biotic reactions. In the previous experiment involving the cylindrical compost, atrazine and chlorpyrifos dissipation was enhanced with increased compost temperatures. However, it was difficult to discern how much of that enhanced dissipation was due to enhanced volatilization. In this experiment, compost air passed through the polyurethane foam traps, which were taken for pesticide residue analysis. As shown in Figure 29, there was some volatilization of each of the pesticides. The cumulative amount of chlorpyrifos recovered was greater than atrazine, which corresponds to their respective vapor pressures of 2.53 and 0.037 mPa (25 and 20 ºC, respectively) (Howard, 1991). Malathion was less volatile than chlorpyrifos with a vapor pressure of 1.03 mPa (Howard, 1991). Malathion was initially detected in the volatile
Figure 28  Pesticide Dissipation in One Cubic Meter Compost

Horizontal bars are standard errors of the means
Figure 29  Pesticides Recovered in Polyurethane Foam Volatility Traps in One Cubic Meter Contained Compost
traps, however, malathion degraded quickly in the compost, which would explain why malathion was not detected in the PUF volatile traps after 2 weeks.

The total amount of the pesticides recovered in the polyurethane foam traps (PUF's) over one month was minimal when compared to the overall amount applied to the compost. The total amounts of atrazine and chlorpyrifos applied to the compost were 15.8 and 33.7 g, respectively. Nearly 1 mg of chlorpyrifos was recovered in the PUF's over the 37 day period, which was negligible at 0.003%. There was some volatilization occurring, but comparatively insignificant. It would have seemed that more chlorpyrifos would have been recovered in the PUF's.

The pressing question was why the chlorpyrifos recovery was lower than expected in the volatile traps. The PUF's were efficient in trapping the pesticides as determined during the cyclohexane extraction. The PUF plugs were segmented, and all of the atrazine, chlorpyrifos, and malathion was recovered in the first couple of centimeters. It was assumed that extraction recoveries were good in this study, since other recovery experiments performed involving 14C-labeled diazinon and chlorpyrifos resulted in near 100% recoveries in organic solvent extractions. The compost boxes were airtight except for the bottom and the top vents. The flow of the air was confirmed to be escaping from the top vents. It is possible that volatilized chlorpyrifos may have sorbed to the compost boxes in void space above the compost matrix. Analytical means were not carried out to confirm chlorpyrifos sorption to the box.

To further determine the fate of volatile pesticides in the enclosed system, a sample of untreated compost was taken at day 7 from the compost overlying the mesh cylinder containing the atrazine, malathion, or chlorpyrifos. No malathion and only trace amounts (<1 µg/g) of atrazine were detected in the overlying compost. However, in the chlorpyrifos compost, an approximate 200 g sample (dry weight) contained 120 µg/g chlorpyrifos, which is approximately 24 mg of chlorpyrifos. It was likely that more chlorpyrifos was associated with more of the matrix overlying the mesh cylinder. Apparently, chlorpyrifos was condensing in the cooler overlying regions of the compost matrix. It is difficult to conclude what percentage of chlorpyrifos did volatilize away from the pesticide core and then condensed in the overlying compost matrix.

The compost overlying the mesh cylinders in all composts was left uncovered for a week after the day 7 sampling. The purpose was to determine if larger amounts of pesticide could be recovered in the PUF volatile traps. There was an increase in cumulative pesticide recovered in the PUF's for all pesticides (Figure 29) during the week
period because there was no overlying compost for condensation to occur. After day 13 the mesh cylinders containing the pesticides were covered with untreated compost for the remainder of the experiment. Even with the pesticide left exposed to the atmosphere, there was relatively little of any of the pesticides detected in the PUF volatile traps. Only slightly detectable amounts (ppb range) of chlorpyrifos were detected in the condensate on the inside of the compost structure's top, which did not amount to much on a relative scale to what was applied. Therefore, it appears that some pesticide volatilization did occur, some of the volatilized chlorpyrifos was found condensed in the cooler regions of the compost, and greater than 99% of the pesticides applied remained inside the compost container. Between 15 and 20% of the chlorpyrifos applied as $^{14}$C radiolabelled chlorpyrifos to lignocellulosic bioreactors (Project VI in this Dissertation) at 40 °C was recovered in PUF's. Depending on the vapor pressure of the pesticide and conditions, upwards of 90% loss by volatilization has been reported in field applications (Taylor, 1978). Trifluralin is considered to be a volatile pesticide (15 mPa, 20 °C) (Taylor and Spencer, 1990) and a 26% loss by volatilization was reported after 9 days and 90% loss by volatilization after 35 days (White et al. (1977). It is likely that volatilization was occurring in the compost, since temperatures in the compost reached approximately 65 °C early in the experiment (Figure 27). Because of apparent condensation, volatilization was not a path of dissipation from the system. Pesticide volatilization may account for more of the overall pesticide dissipation when the entire compost matrix has pesticide associated with it as would be the case in a full scale pesticide waste disposal system in operation.

4.3.5 Conclusion

From the results of field compost trials, some recommendations of different compost structures and management practices were formulated to ensure optimal compost conditions and subsequent maximized rates of pesticide dissipation. It is important to maintain compost at temperatures exceeding 50 °C to increase decomposition, which include the abiotic and biotic mechanisms of pesticide dissipation in compost. Faster rates of pesticide dissipation are paramount in a biologically based system designed to detoxify waste pesticides.

To achieve the higher compost temperatures exceeding 50 °C needed for optimal decomposition, certain criteria should be met. It was apparent from this study that good
aeration of the compost is essential to maintain oxygen levels above 7%, which has been reported to be the minimum oxygen concentration for optimal microbial activity and growth (Mathur, 1991). Therefore, bulking materials, such as peanut hulls or wood chips, should be mixed into the compost. Bulking materials are especially important if fresh grass clippings are used, because grass clumps together in compost and does not have adequate airspace.

Because the compost materials used in the pesticide waste disposal system are primarily peat moss and steam-exploded wood and peanut hull products, supplemental nitrogen and easily metabolized energy sources need to be added to the compost. Peat moss and the steam-exploded products have a fairly high C:N ratio exceeding 50:1. In one instance during this study, compost temperatures dramatically increased with only chicken manure added as a source of nitrogen. Apparently, the presence of nitrogen may be as important if not more important than adding supplemental energy sources, such as corn meal.

The importance of adding nitrogen supplements to a peat moss and steam-exploded products compost may not supersede the need to amend with additional sources of easily metabolized energy sources, such as corn meal, vegetable oil, or grass clippings. It was obvious that compost temperatures do dramatically increase with additional energy sources. It was interesting to find that vegetable oil received as waste from a fast food restaurant and added to compost provided high compost temperatures. The high temperatures were attributed to the good coverage throughout the compost and that lipids have approximately twice the metabolic energy as similar amounts of carbohydrates. Grass clippings are a good energy source and also provide nitrogen to the compost. Grass clippings should be included in the normal construction of the compost used to detoxify pesticides.

The compost size should be approximately one cubic meter, which is related to how well the heat generated by microorganisms is held within the compost. Too much heat can be lost in compost with a size much less than 1 m³ (Golueke, 1972; Polprasert, 1989; Biddlestone et al., 1987; Mathur, 1991). If the compost cannot retain heat, the beneficial thermophilic microorganisms will not thrive. It is the thermophilic stage which appears to be most important in organic material decomposition, which includes pesticide decomposition. The observed compost temperatures exceeding 65 °C in the cylindrical and cubic meter compost units support the concept of minimal compost size.
There is a disadvantage of increasing the compost size to a cubic meter. The larger compost volume requires considerable time to mix compared to the smaller volume in the 189 L bioreactors. Compost temperatures of 50 °C are achievable in the 189 L bioreactors if bulking materials are used to promote aeration and supplemental sources of nitrogen and energy are added. The fact that the 189 L compost units are sunken into the ground and contained in a safety liner are advantages to this system. The potential of leachates from any of the above ground compost units used during this study would require the construction of a concrete pad or catchment under the compost units.

At some point during the management of compost used to detoxify pesticides, there would be a need to remove the mature compost with low levels of pesticides for new batches of pesticide waste. It may be difficult to take larger amounts of mature compost to some final resting place, especially since it could still be considered a hazardous waste. Specific transportation and disposal laws have to be followed in dealing with material classified as hazardous waste. If it is to be incinerated or landfilled, greater costs will be incurred with larger amounts of material taken for disposal. It is not as much of a problem if the spent compost can be land applied. However, the Resource Conservation and Recovery Act requires that the person or persons disposing any hazardous waste must petition the EPA for a permit. The EPA has to be assured that the waste being disposed of does not pose a threat to groundwater or ecosystems. It would seem that land application is a viable option if the spent compost that is to be spread on land has low concentrations of pesticides that would be applied under normal pest control management.
4.4 PROJECT IV The Effect of Vegetative Fragmentation and Mixing by Mite Activity on Atrazine Dissipation in Compost

4.4.1 Abstract

Mechanical fragmentation and mixing of vegetative detritus by invertebrates and its importance in compost maturation is an important contribution to the decomposition process. The main focus of these experiments was to determine if vegetative fragmentation and mixing by mites (Acarina) in compost increased atrazine degradation in a compost bioreactor designed to degrade waste pesticides. Additional treatments were studied to determine the effect on mite populations, which included hydration levels of the compost, adding corn meal, and freezing the compost.

There were five major species of mites found in a field compost composed of steam-exploded wood (yellow poplar). A mite species (approx. 0.5 mm) was classified to the family Ologomasisidae were predaceous on nematodes and other species of mites. The other four different mites observed in the compost were grazers, possibly feeding on fungi and bacteria. Two of the grazer mite species appeared to be mycophagous; one species with a tortoise-like shell (0.4 mm), which was identified to the superfamily Nothroidea, and the other species was globular and oval (0.1 - 0.2 mm), which was identified to the family Uropodidae. Another mite species was globular and oval (0.5 mm), which was identified to the family Acaridae. The feeding behavior and mouthpart structure indicates that the Acaridae species was microphytophagous. The last mite species described was small and oval (<0.1 mm), which was identified as an Acaridae hypopoid deutonymph. It may be that this hypopoid deutonymph was the phoretic stage of the adult Acarid identified, since both were present and no other Acaridae adult was found.

The population or numbers of mites for all species were greatest at the beginning of the experiment (Week 2). The numbers of all species declined over the five week period and dehydration in the compost may have been the reason. Populations of the Acaridae deutonymphs and adults were significantly larger in the 70% hydrated compost samples (H₂O/wet compost; w/w) compared to the 50 and 30% hydrated samples (ANOVA, α =0.05). The Nothroidea species and the predaceous Ologomasisidae species appeared to have significantly higher populations in the drier 30% compost (ANOVA, α=0.05).
The populations of mites in compost amended once with corn meal versus compost amended again after one month were not significantly different (Student's t-test; $\alpha = 0.05$). However, general trends indicated that given more time and replicates, mite numbers would have proven to be higher in compost amended twice with corn meal.

One objective of the study was to eliminate mites from the compost samples in future experiments that were designed to determine what role microbes have in the degradation of pesticides in compost. There were no chemicals to control mites added to compost to prevent positive or negative effects on the microbes present. Freezing (-15 °C) compost for 48 hours and 1 week durations was examined as a physical control method, which resulted in no emergence of mites after 4 weeks of incubation (21 to 28 °C) in compost originally containing a mite population. Freezing did not appear to disrupt fungal growth in control samples compared to samples not frozen.

The presence of mites in compost had a profound effect on enhancing atrazine degradation in the compost test system. After 12 weeks of incubation with an initial atrazine concentration of 7500 µg/g dry matrix, atrazine concentrations of 3254 ± 114 µg/g in mite-infested compost were significantly lower ($P = 5.03 \times 10^{-5}$) than 4504 ± 119 µg/g atrazine in compost without the presence of mites.

4.4.2 Introduction

Mites are part of the normal fauna in compost under many circumstances. Their presence is important in the degradation and maturation of the healthy aerobic compost pile (Minnich, 1979).

Mites can create a large problem in many agricultural and horticultural situations, and there has been many research topics involving population dynamics, sampling, and control of mites in these economic areas. The presence of mycophagous mites in cultivated compost can create a control problem in the mushroom industry because mature compost is used for the growing substrate (Krantz, 1978). Some typical mycophagous mites pests encountered in mushroom production are *Typhagus putrescentiae* (family: Tyroglyphidae), *Linopodes antennapes* (family: Eupodidae), *Tarsonemus myceliophagus* (family: Tarsonemidae), and *Pygmeophorus* sp. (family: Pyemotidae) (Stamets and Chilton, 1986). On the other hand, the presence of mites can considered to be beneficial in compost used purely to degrade organic material and reduce volume. It has been shown
that members of the family Orbatidae can accelerate cellulose and lignin degradation (Scheu, 1992; Schultz and Scheu, 1994). Most research has focused on the role that microinvertebrates have in decomposing litter. Few reports of mite ecological behavior and population dynamics exist for compost.

Biological activities of compost or aerobic solid state fermentation technology are currently being used as means to degrade waste pesticides (Mullins et al., 1990). Their research has shown that high concentrations of waste pesticides (>20,000 µg/g) can be degraded in compost through chemical and biological influences. Mites appear to play an integral role in the maturation of compost (Polprasert, 1989), therefore their role in pesticide degradation may be important. Several mite species are mycophagous (Alexopoulos and Mims, 1979). Mites have been observed in laboratory and field studies with solid state fermentation bioreactors, and it appears their population growth is quick.

Mite species, their biology, their ecological role, population dynamics, and the effect they have on the success of pesticide degradation are areas that these experiments address. Specifically, the population dynamics of the mites were examined in accordance with varying compost hydration (70, 50, & 30%; H₂O/wet compost, w/w) and subjection to freezing temperatures (-15 °C) for two durations (48 hours and 1 week). It was believed that greater hydration levels would facilitate mite growth due to better conditions for microbial growth and activity. One goal was to determine if freezing the compost collected from the field and containing mites would eliminate them from emerging later on during subsequent use of the compost material. Controlling mites was of particular interest, because the presence of mites (and their metabolic output) in other experiments (not addressed here) have confounded respirometry work used for determining microbial activity in compost. It was believed that mites in compost being used in laboratory experiments would be controlled by freezing the compost for some duration at -15 °C without affecting the ability for microbes to grow. The addition of chemicals to control mites was not done in order to avoid any deleterious or stimulatory effect on the microbial population.

In addition to the objectives listed above, mite populations were compared with treatments of increased microbial activity in the compost by amending the compost with corn meal once, twice, and not at all. It was thought that amending corn meal to the compost would increase the biological activity, including that of mites, involved in compost maturation.
The final objective determined the influence that mites present in compost would have on enhancing pesticide dissipation. It was believed that because the presence of mites has been shown to enhance litter, cellulose, and lignin decomposition, their presence would also enhance pesticide degradation.

4.4.3 Materials and Methods

Materials

Analytical grade 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-s-triazine (atrazine) (>99% purity) and formulated atrazine (AATrex® 4L) were supplied by Ciba-Geigy Corporation (Greensboro, NC). Unless otherwise specified, chemicals were reagent grade and solvents used were at least pesticide grade; these were purchased mainly from Fisher Scientific (Atlanta, GA).

Mites

An inoculum of mites was collected from field and laboratory compost located at the Turf Grass Farm and the Department of Entomology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia. Approximately 0.5 L of the compost inoculum was mixed in an approximate one liter Mason® jar, and a 25 mL subsampie was added to the appropriate treatments, and mixed well before water was added. It was believed that mixing the compost did not harm the mite inoculum added to the matrix.

Compost and Treatments

The compost was constructed using steam-exploded wood or SEW (yellow poplar) obtained from the Forestry Department at Virginia Tech. To each approximate 500 mL Mason® jar, 10 g of dry SEW was added with the mite inoculum. Tap water was added to the jars in the appropriate amounts to make hydration levels of 70, 50, and 30% water/total compost weight (w/w). Ground cornmeal (2 mm or 10 mesh) was added at 0.5 g/jar to the appropriate treatments (all except for the not amended controls), and mixed well. Aliquots of the formulated atrazine as AATrex® 4L were mixed into one set 50% compost jars to result in an approximate initial atrazine concentration of 5000 µg/g dry weight matrix.
Incubation

Jars were topped with brown paper towels secured with the jar ring. The paper towel was used to keep insects and mites contained while allowing sufficient oxygen transfer. Jars of compost were kept in a steel cabinet in cardboard boxes with a drugstore heating pad set on high to heat the incubator. The internal temperature was taken daily and ranged from 21 to 28 °C.

Sampling

At appropriate times, jars were removed from the incubator and the contents, jar, and paper top were placed into a Berlese funnel for 24 hours. The organisms found in the 20 mL of 70% (v/v) ethanol at bottom of the Berlese funnel were capped with a solid top for examination and enumeration at a later date.

Estimating Population Number

The specific species of organisms in each jar were enumerated by pouring the contents into a 120 mm plastic Petri dish which had 8 radially equal divisions marked from below. Rinsing the side of the jar was necessary in certain circumstances to remove as many mites as possible. The 70% ethanol solution in the Petri dish was then removed by glass pipette, being careful not to remove any mites. Descriptions and numbers of the different types of mites present were recorded for the Petri dish and then poured back into the jar for storage.

Experimental Design

There were four objectives in this study. The first objective involved examination of the effect that hydration has on mite succession and population dynamics, and involved three replicates of 30, 50, and 70% compost hydrations (weight of H₂O/weight of wet compost matrix). Corn meal was added only at the beginning of the experiment.

The second objective involved examination of the response of the mites to the compost being amended with corn meal once at experiment initiation, the compost being amended twice (once at the beginning and another amendment after approximately three weeks), and compost not amended at all. The compost hydration level was initiated at 70%.

The third objective was to study the effect that freezing compost (-20 °C) for either 24 hours or 1 week had on controlling the emergence of mites from the compost.
The fourth objective was to determine the effect that the presence of a mite population had on enhancing atrazine degradation. All treatments had an approximately 5000 µg/g atrazine and included controls frozen (-20 °C) at experiment initiation, sterile controls (without mites), and samples with inocula of mites. Amounts (5 g) of corn meal were added at the beginning of the experiment similar to the hydration portion of these experiments. Atrazine-treated samples were not sacrificed to count mites, but observations of general mite populations were taken. All samples were frozen (-20 °C) after 8 weeks for analysis of atrazine levels.

**Statistical Analysis**

Analysis of variance (ANOVA) (α = 0.05) was performed on the data to test for significant mite population differences between the time (weeks 2-5) or treatments (70, 50, 30% hydration). Duncan's comparison of means was performed (α = 0.05) for each separate class variable when significance was evident in ANOVA with the class variables.

**Extraction of Matrix**

The contents of each bioreactor was mixed well by hand and the weight of matrix recorded. The ratio of wet vs. dry matrix from each bioreactor was determined by transferring approximately 10 g of the wet matrix into 1.0 g aluminum weighing dishes, recording the wet matrix weight, drying the matrix at 110 °C for 48 hours, and recording the dry matrix weight. Based on the wet/dry ratio, weighed proportions of the wet matrix approximating 1.0 g dry matrix were transferred to 40 mL polypropylene centrifuge tube (Fisher Scientific). Fifteen mL of ethyl acetate was added to each centrifuge tube containing the matrix, the contents shaken by hand for one minute, then allowed to sit overnight, not exceeding 12 hours. The centrifuge tubes were then placed into a sonicating water bath (Fisher Scientific) for three separate 30 minute periods with 30 minute intervals of not sonicating. The contents were centrifuged at approximately 3000 rpm (1000 x g; Beckman Centrifuge, Beckman Instrument, Inc., Irvine, CA) for 15 minutes. The supernatant was decanted into 500 mL beakers, which were placed in a fume hood to reduce the volume. The extraction process was repeated for a total of three times, repeating the addition of 15 mL of ethyl acetate, the sonication process, the centrifugation, and decanting the supernatant into the same 500 mL beaker. After the fourth extraction the matrix was allowed to dry before being stored at -20 °C. The volume of the collective supernatant from each bioreactor was reduced to approximately
25 mL in a fume hood and then adjusted to 30 mL with ethyl acetate. Ten mL aliquots were taken and stored at -20 °C for future chromatographic analysis.

Previous recovery experiments involving spiking small amounts of matrix (2 g dry) with 1 mg atrazine were quite variable and dependent on the amount of time allowed before extraction. Atrazine recoveries using the above extraction procedures were 52 to 87%. The 52% recovery resulted from samples allowed to sit at room temperature for 48 hours before extraction.

**Analysis of Extract**

A 1:100 dilution of the stored solution was made with ethyl acetate and 2 μL aliquots of the extract were injected into a Tracor 540 gas chromatograph with a 6 ft (1.8 m) glass column (1/4 inch or 6.35 mm inner diameter) packed with OV-17/OV210 (1.5%/1.95%) (100 mesh/120 mesh) Chromosorb® WHP equipped with an electron capture detector. Instrument temperatures were: injection port, 225 °C; column, 180 °C; detector, 350 °C; and carrier gas flow rate, 35 mL/min N₂. Standards of atrazine ranging from 0.001 to 10 ng/μL in ethyl acetate were kept in 4 mL glass vials and 2 μL aliquots of the standards were injected onto the column delivering 0.002 to 20 ng for a standard curve. The average retention time for atrazine in this chromatographic system was 4.5 minutes. The 0.002 ng atrazine standard gave a good electron-capture detector response, therefore the instrument limit of detection (LOD) was well below 0.002 ng. The LOD in the experiment was based on the 0.002 ng standard and the volume of the extract, the LOD for atrazine was 10 μg/g dry matrix (ppm).

4.4.4 Results

The 70% hydration level was added to the study in place of a 90% level because the initial 90% jars were oversaturated with water. The amount of water added to result in 90% water compared to the wet weight of the matrix was more than the matrix could absorb. Over the five week period, all 50 and 70% compost jars lost approximately 20% of the original water amount. The 30% jars appeared to remain approximately 30%.

The effect of this water loss on mite behavior and population dynamics is not evident. The paper towel top was effective for keeping mites and insects in the jar, but did not appear to impede water loss. In future studies, waxpaper may be better for preventing
water loss, and porous enough to allow adequate gas transfer. Adding water as the compost dries may be another way to keep original hydration levels somewhat consistent.

The steel cabinet transformed into an incubator was not insulated, which may explain why temperatures fluctuated between 21 and 28 °C over the five week period. Additional studies should involve higher and more constant temperatures. Ideally, the incubation temperature should be similar to field conditions, which usually exceed 30 °C.

Berlese funnels were used to collect mites and other compost organisms. The Berlese funnel method for collecting mites was easy and effective. The recovery of compost mites was nearly 100%, since on inspection of dried compost after 24 h, no dead mites were found. In a 30%-5th week sample, 3320 newly emerged individuals were found in the alcohol (Table 17). These mites were over five times smaller (<0.1 mm) than their larger counterparts. Some species were slower than others, but all were mobile enough to leave the compost in the Berlese funnel and fall to the ethanol trap below.

A flotation method was examined for collecting mites by covering the compost with water and collecting floating mites. This process was not successful because the mites did not release from the submerged compost. They crawled over the compost as if the water was not present. Around each mite was an air pocket, most likely created by their hydrophobic exoskeleton and several external hairs. This morphological adaptation may help soil or detritus mites survive wet conditions.

A 120 mm Petri dish divided into 8 radially equal segments was used as a counting dish. Counting at approximately 10 X magnification was best when almost all of the liquid was removed by glass pipette and placed back into the jar. The liquid removed prevented the mites from moving into other sections of Petri dish as it was rotated during counting. Removing the liquid was done without removing mites by keeping the glass tip of the pipette close to the Petri dish while suctioning the liquid. Any mites left in the jar or removed by pipette were counted in the jar and added to the final total numbers. In the future it is recommended to have the 8 sections in the Petri dish blocked from one another by walls. Counting could be done quickly, without spending the time to remove the liquid, and ensure that mites do not spill over into adjacent sections.

Mites in the Compost

Throughout the five week period several species of organisms were noticed in various amounts and combinations. Present in most jars in greater or lesser amounts were
Table 17  Number\textsuperscript{1,2} of Mites Estimated in Compost as Influenced by Compost Hydration Amounts of 70, 50, and 30% Water\textsuperscript{3} (w/w)

<table>
<thead>
<tr>
<th>Hydration</th>
<th>Mycophagous</th>
<th>Mite Species\textsuperscript{4}</th>
<th>Micophytophagous</th>
<th>Predaceous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acaridae\textsuperscript{5}</td>
<td>Ologamidae\textsuperscript{6} (adult)</td>
<td>Ologamidae\textsuperscript{6} (juvenile)</td>
<td></td>
</tr>
<tr>
<td>Amount\textsuperscript{7}</td>
<td>Week</td>
<td>Nothroidae\textsuperscript{8}</td>
<td>Hydropoid deutonymph\textsuperscript{9}</td>
<td>Acaridae\textsuperscript{6}</td>
</tr>
<tr>
<td>70%</td>
<td>2</td>
<td>4 ± 1.5</td>
<td>764 ± 227</td>
<td>1236 ± 249</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1 ± 1</td>
<td>592 ± 41</td>
<td>481 ± 64</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10 ± 6</td>
<td>198 ± 54</td>
<td>76 ± 30</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>17 ± 5</td>
<td>674 ± 390</td>
<td>0</td>
</tr>
<tr>
<td>50%</td>
<td>2</td>
<td>7 ± 2</td>
<td>40 ± 28</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3 ± 2</td>
<td>100 ± 43</td>
<td>7 ± 7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11 ± 6</td>
<td>54 ± 34</td>
<td>41 ± 25</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7 ± 5</td>
<td>3 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>30%</td>
<td>2</td>
<td>8 ± 4</td>
<td>322 ± 173</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18 ± 1</td>
<td>234 ± 45</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13</td>
<td>54 ± 28</td>
<td>44 ± 10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Mite population determined by Berlese funnel and numbers of mites determined from three replicates per week per treatment

\textsuperscript{2}Numbers of mites are presented as means \pm standard error of the mean

\textsuperscript{3}Hydration amount is based on water weight/wet matrix weight

\textsuperscript{4}Mite species identified to Superfamily or Family

\textsuperscript{5}Hydration amount is based on water weight/wet matrix weight

\textsuperscript{6}Nothroidae Superfamily

\textsuperscript{7}Acaridea family (hypopoid deutonymph)

\textsuperscript{8}Acaridea Family

\textsuperscript{9}Uropodidae family

\textsuperscript{10}Ologamidae Family (adult)

\textsuperscript{11}Ologamidae Family (juvenile)
mites and fungi. Other inhabitants included nematodes, springtails (Collembola), thrips (Thysanura), flies and fly larvae (Mycetophilidae), and spiders (Araneae).

There were at least five major species of mites found frequently in the compost during this study. Most mites could be seen with the naked eye and all could be observed with a 10X magnification hand-lens. One species was oval (0.3 x 0.4 mm) and had a tortoise-like shell, which was classified to the Superfamily Nothroidae. Species in this Superfamily are common cosmopolitan litter habiting mites (Kranz, 1978).

The adult of another species can be described as long (0.5-0.75 mm), slightly oval, and globular in shape (as tall as wide; 0.3-0.5 mm). These were classified to the family Acaridae. A possibly related mite was observed, which were small (0.1 mm) and had two distinct dark half moon-shaped marks on the lateral edges of the abdomen and were visible from above the mite. These mites were identified as hypopoids of the family Acaridae. Hypopaeae are the phoretic deutonymphal stage which usually occurs under adverse habitat conditions. They have short legs and a sucker plate that allows the hypopae to attach themselves to passing invertebrates, such as beetles and flies. Once attached they are transported elsewhere, being passively dispersed to possibly better soil and detritus conditions. In one circumstance in a composting container, these phoretic mites were observed to attach onto a beetle, covering it to the point where it could hardly move. The hypopoid deutonymphs may have been related to the adult Acaridae species found in the compost, since both mite types were present and no other Acaridae adults were present.

There also was a smaller globular species (<0.1-0.2 mm), which was classified to the family Uropodidae. Species in the Uropodidae are commonly found in forest litter and detritus, as well as soil, moss, rotting wood, and insect nests and galleries (Kranz, 1978).

There was a flattened species that had a leathery dorsal abdomen and was observed to be predaceous on other mites and nematodes. The larger adults (0.5 mm long by 0.25 mm wide) and smaller morphs (0.2 mm long by 0.1 mm wide) were classified to the family Ologamasidae. These are commonly found predators in soil, humus, and compost (Kranz, 1978).

The behavior of the five major species of mites was observed. Many individuals of this species were found on top of the compost, but were apparently in the same numbers throughout the compost where cracks and crevices were available. None appeared to burrow under the compost substrate. The Nothroidae adults and Acaridae hypopae were quite mobile and could move several centimeters within minutes. However, the Acaridae and Uropodidae adults were lethargic, moving comparatively slowly and sporadically.
The Nothroidea, Acaridae and Uropodidae adults, and Acaridae hypopae appeared to be grazers in the compost. Much of the time these species were seen probing through wet and dry areas of the compost. They may have been feeding on fungal mycelia and/or ingesting nutritive liquid that was pooled. It is important to note that fungal mycelia and fruiting bodies were minimal in compost jars with the grazer mites species present, but were prolific in jars with no or few grazer mite individuals. Similar moisture conditions were present in compost with the grazer mites versus no grazer mites.

Individuals of the predaceous Ologamasidae species were quite mobile, and in most circumstances did not stop moving. Within seconds they could move out of the field of view (approximately 3 cm). They appeared to be somewhat predaceous, though not voracious. After watching their activities for two different 30 min intervals among the slower grazers, one Ologamasidae individual was observed attacking and consuming an Acaridae hypoid. They frequently probed or palpated many mites, including each other, but were not observed attacking other mites that often.

Several Ologamasidae individuals were placed into a separate compost jar containing no other Ologamasidae mites and many nematodes slithering through the soft liquid substrate. Within minutes, several Ologamasidae individuals were observed attacking and consuming nematodes. Jars that had numerous Ologamasidae individuals did not appear to have nematodes on the immediate substrate surface, but were easily observed in similar jars without Ologamasidae mites.

Effect of Compost Hydration on Mite Population Profile and Dynamics

In the hydration study, mites began to appear between the first and second weeks, and most of the species had the greatest numbers at the second week mark (Table 17). There were significant differences of mite population densities between hydration levels over the 5 weeks for all species and their morphs (Acaridae hypopae, P = 0.0005; Nothroidea, P = 0.043; Acaridae adults, P = 0.0005; Uropodidae, P = 0.007; Ologamasidae adults, P = 0.014; Ologamasidae juveniles, P = 0.01). Using Duncan's comparison of means (α =0.05), it was determined that the means of mite numbers in the 70% hyrdated samples were significantly greater than the other hydration level means for the Acaridae hypopae, Acaridae adults, and Uropodidae mites (Table 17). However, the mean number of mites present in the 30% hydrated samples were significantly greater for the Nothroidea and Ologamasidae species. The means of Ologamasidae adult mite
numbers in the 70 and 30% hydrated samples were significantly greater than in the 50% samples.

It should be noted that the compost in the 50% hydrated samples had a quick fungal growth, and many individual mites were observed dying from the fungi. Many slow moving mites were granular white, which may have been mycelia growing inside the body. The 30% hydrated samples appeared to be too dry for much fungal growth, and the 70% hydrated samples may have had fungus eaten by the large bloom of mites between the first and second weeks.

It appears that the population dynamics of different species is different under varying hydration levels (Table 17). The Acaridae hypopae were numerous early, decreased, and then had a resurgence in numbers at week 5. The Nothroidea species were always present, but only increased slightly to week 5. The Acaridae and Ologamasidae adults were quite numerous during weeks 2 and 3, but their numbers diminished in later weeks. The densities of the predaceous mites were never high, but they appeared to increase as the weeks passed (Table 17).

The Acaridae adult population appeared to diminish after the first four weeks, and reasons may have involved hydration levels (Table 17). All 70 and 50% hydrated samples dried considerably (approx. 40%) during the five week period, which may have been detrimental to optimal growth. They appeared to require greater hydration levels because they were present after seven weeks in compost jars containing atrazine, which were quite hydrated (>70%).

Large numbers of the predaceous species were only observed in the 30% hydrated samples (Table 17). There were relatively numerous predaceous individuals by week 5 in the 70% hydrated samples, which had dried considerably (between 40 & 50% hydration). The predaceous species was also numerous in the samples amended twice (Table 18), and after 5 weeks those jars were quite dry. In fact, in all samples that had dried out considerably, the predaceous species was the sole species or the preeminate one. They appeared to be predaceous on nematodes and other mite species.

The relation between the predaceous species and decreases in other mite species in the compost is unclear. Also, it is not known if the disappearance of the Acaridae and Ologamasidae adults is related to hydration levels or some amount of predation by the predaceous species. Both the Nothroidea species and the Acaridae hypopae increased over time even though the compost dried (Table 17). They may tolerate a broader range of moisture than the Acaridae adults.
Amending Compost with Corn Meal Once, Twice, or Not At All

There were practically no mites found in 70% hydrated samples not amended with any corn meal at any time (Table 18). The Nothroidea and adult Ologamasidae species were found in the non-amended controls, but in only one samples. The Ologamasidae adults may be able to withstand more Spartan conditions than the other species as indicated by their increase in drying compost.

There were no significant differences between mean mite numbers amended once and twice for all species using Student's t-test (\( \alpha = 0.05 \)). Since only three sample in each treatment were tested, the t-values from a t-test table were high. If the \( \alpha \)-level was changed to 0.1, there were significant differences of mean mite numbers between compost amended once and amended twice in the adult Acaridae and Ologamasidae species (Table 18). There may be a trend of more mites in the amended twice jars compared to amended once as indicated in Table 18. If there had been more sample replication, and a couple more weeks had passed beyond the five week incubation period, there may have been more mites in the amended twice jars. In any case future studies should be done to determine if mite population is significantly affected by feeding corn meal more than once.

Freezing as a Means to Control Mite Emergence or Survival

In both the 48 hour and one week compost treatments at -20 °C there were no mites present after 4 weeks of incubation. Freezing at -20 °C may be an effective means to control mites in compost if they are not desired. Because freezing seems to be detrimental to mite survival and emergence, the question can be raised of how they survive in the field. They may be able to physiologically prepare for freezing temperatures or migrate far enough into the soil to escape such harshly cold temperatures.

Enhanced Atrazine Degradation by the Presence of Compost Mites

Mites were present in all seven compost vessels containing atrazine, and no mites were observed in the 4 compost vessels designated controls without mites. Mite population size was not quantified as was done in previous sections, but it was obvious that their presence and species profile was similar to the 70% hydrated compost vessels mentioned above.

The initial atrazine concentration was determined to be 7437 ± 1388 µg/g dry matrix. After 12 weeks of incubation between 23 and 27 °C, the atrazine concentration
### Table 18  Population\textsuperscript{12} of Mites Estimated in Compost Amended Once, Twice, or Not Amended with Corn Meal\textsuperscript{3}

<table>
<thead>
<tr>
<th>Week</th>
<th>Nothroidae\textsuperscript{5}</th>
<th>Acaridae Hypoophid deutonymph\textsuperscript{9}</th>
<th>Acaridae\textsuperscript{7}</th>
<th>Uropodidae\textsuperscript{8}</th>
<th>Ologamasidae (adult)\textsuperscript{6}</th>
<th>Ologamasidae (juvenile)\textsuperscript{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mite Species\textsuperscript{4}</td>
<td>Mycophagous</td>
<td>Microphytophages</td>
<td>Prodaceous</td>
<td>Mycophagous</td>
<td>Microphytophages</td>
</tr>
<tr>
<td></td>
<td>Compost Not Amended</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3 ± 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10 ± 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Compost Amended Once\textsuperscript{11}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4 ± 4</td>
<td>28 ± 22</td>
<td>1 ± 1</td>
<td>0</td>
<td>57 ± 42</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Compost Amended Twice\textsuperscript{12}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8 ± 7</td>
<td>60 ± 19</td>
<td>51 ± 44</td>
<td>20 ± 20</td>
<td>94 ± 5</td>
<td>138 ± 80</td>
</tr>
</tbody>
</table>

---

\textsuperscript{1}Mite population determined by Berlese funnel and numbers of mites determined from three replicates per week per treatment.

\textsuperscript{2}Numbers of mites are presented as means ± standard error of the mean.

\textsuperscript{3}Hydration amount is based on water weight/wet matrix weight.

\textsuperscript{4}Mite species not identified, but designated by feeding behavior & general appearance.

\textsuperscript{5}Nothroidae Superfamily

\textsuperscript{6}Ologamasidae Family

\textsuperscript{7}Acaridae Family

\textsuperscript{8}Uropodidae Family

\textsuperscript{9}Ologamasidae Family (adult)

\textsuperscript{10}Ologamasidae Family (juvenile)

\textsuperscript{11}Corn meal added once at experiment initiation.

\textsuperscript{12}Corn meal added twice, once at experiment initiation and again after 3 weeks.
of 3254 ± 114 μg/g (mean ± sem) in the mite-inhabited compost vessels were significantly lower (P = 5.03 x 10^{-5}) than 4504 ± 129 μg/g in compost vessels without the presence of mites.

4.4.5 Discussion

It was obvious that mite populations were larger in compost at a hydration of 70% (amount of water/amount of wet matrix) (Table 17). Mites were adversely affected in compost that were 50 and 30% hydrated. Therefore, the experiment studying the effects of mites on the dissipation of atrazine was performed using compost hydrated at 70%. Proper aeration is the only caveat to having compost that is too hydrated. If air cannot penetrate the compost matrix because of water, anaerobic conditions will prevail and invertebrates will move to aerated regions.

The compost environment at 70% hydration appeared to be quite diverse with mites, nematodes, fungi, bacteria, and other organisms. Figure 3 (Chapter 1, Introduction) illustrates the ecological food web typical in compost. It has been demonstrated that soil inhabiting arthropods are the primary regulators of loss of litter mass (Santos and Whitford, 1981; Seateldt and Crossley, 1983). Several workers have shown that the action of vegetative fragmentation and mixing has profound effects on increasing the mass loss of vegetative material in soil (Scheu and Wolters, 1991; Siepel and Maaskamp, 1994; Vikram et al., 1994). The invertebrates' feeding activity acts to fragment vegetative matter in a similar manner as aquatic invertebrates do in shredding the vegetative material input into streams, rivers, and lakes. The action of fragmenting vegetation by invertebrates creates more surface area on which fungi and bacteria can grow, which further increases metabolism and degradation of the vegetative litter or detritus. It stands to reason that if a pesticide is susceptible to biodegradation, bioturbation will enhance the pesticide biodegradation by increasing overall microbial activity.

There were considerable numbers of mites grazing in the compost, feeding primarily on fungi. Van der Drift and Jansen (1977) and Hanlon (1981) demonstrated that grazing on fungi is the predominant feeding behavior of mites found in vegetative decomposition. Grazing by mites in this study accelerated degradation of atrazine. In addition to compost matrix fragmentation, the activity of mites feeding and defecating may
also have been responsible for the enhanced atrazine dissipation. Increased microbial activity may have occurred with the feeding activity of the mites. It is unclear what impact that digestive enzymes may have had in degrading the atrazine. It is likely that some atrazine passed through the mites as they fed on fungi and bacteria. Additionally, the fecal material may have increased microbial activity. There was an obvious difference between the 70% hydrated compost samples with and without mites. The compost infested with mites was covered by a black material that looked like the outer top layer had been charred. It would seem that atrazine dissipation may have been affected by the digestion of mites in compost.

The overall combination of invertebrates (mites in this particular study) and the increase of bacteria and fungi due to the vegetative fragmentation and mixing of the matrix is a healthy and stable situation from an ecological point of view, as well as beneficial for degradation of pesticides in compost. It would be desirable to maintain composting conditions such that mites and other invertebrates can perform their ecological role in the decomposition of vegetative material. Atrazine dissipation was enhanced by the presence of mites in compost, and evidently atrazine and the chemicals used in the atrazine formulation were not toxic to mites. However, other pesticides, specifically insecticides and miticides, may adversely affect mites, reducing their role in vegetative decomposition and enhancing pesticide dissipation. In conclusion, it appears that the fragmentation and mixing activity by mites and other invertebrates are important to consider in managing compost designed for degrading pesticides.
4.5 PROJECT V  Effects of Nutrient Amendment on Atrazine and Chlorpyrifos
Dissipation in an Aerobic Composting System Employing
Lignocellulosic Materials Designed for Pesticide Waste Disposal

4.5.1  Abstract

Experiments were conducted to observe the effects that amending lignocellulosic-
based bioreactors with nutrients had on the microbial activity and dissipation of atrazine or
chlorpyrifos, both initially at approximately 5000 μg/g. The bioreactor matrix was a 1:1:1
mixture (w/w; dry weight) of peat moss, steam-exploded wood (SEW), and steam-
exploded peanut hulls (SEPH).

The addition of nutrients significantly increased overall microbial activity indicated
as oxygen consumption rates, which ranged from 50 to 400 μL/minute in bioreactors not
amended with corn meal or vegetable oil and from 50 to approximately 2350 μL/minute in
bioreactors amended with corn meal or vegetable oil. The profile of oxygen consumption
rates or microbial activity was similar between bioreactors amended with corn meal and
bioreactors amended with vegetable oil. Mixing the bioreactors approximately two weeks
after nutrient amendment increased microbial activity, most likely due to a redistribution of
nutrients and microbial populations. Towards the end of the experiment, the microbial
activity decreased and did increase after approximately 25 days from the time nutrients
were reapplied. It was concluded that the lack of microbial activity was not due to
toxicity, since the same effect was observed in bioreactors without pesticides. The only
significant pesticide inhibitory effect on microbial activity was a slight inhibition
(P = 0.047) of oxygen consumption rates in corn meal amended bioreactors with
approximately 4750 μg atrazine/g dry matrix (ppm).

After 16 weeks at approximately 45 °C, the concentration of atrazine declined to
approximately 0.3% of the initial 4750 ± 419 μg/g (+ standard error of the mean) for all
treatments. The DT₅₀ values for atrazine dissipation after 16 weeks were calculated to be
approximately 0.7 weeks (r² = 0.96) in corn meal amended bioreactors, 1.0 weeks (r² =
0.96) in vegetable oil amended bioreactors, and 1.1 weeks (r² = 0.96) in the bioreactors
not amended. The presence of corn meal appeared to significantly enhance atrazine
dissipation at 4 weeks, there were no significant differences in dissipation after 16 weeks in any of the bioreactors.

Converse to the atrazine bioreactors, chlorpyrifos dissipation after 16 weeks increased significantly upon the addition of nutrients. The chlorpyrifos concentration declined to 2.25, and 17% of the initial $4569 \pm 274 \mu g/g$ in bioreactors amended with corn meal, vegetable oil, or not amended, respectively. The $DT_{50}$ values for chlorpyrifos dissipation were calculated to be approximately 1.7 weeks ($r^2 = 0.99$) in corn meal amended bioreactors, 8.9 weeks ($r^2 = 0.98$) in vegetable oil amended bioreactors, and 6.2 weeks ($r^2 = 0.99$) in the unamended bioreactors.

4.5.2 Introduction

The proper disposal of pesticide waste is an important concern from a human health, wildlife, and environmental aspect. Pesticide waste may include excess or outdated concentrate, excess amounts left after application, and rinsates that may include the rinsate after washing equipment, as well as pesticide containers.

Some of the common recommendations for disposal of pesticide waste include container recycling (Fitz, 1992), landflling within the constraints of Federal, State, and local laws, and containment of rinsates with further disposal. Methods of further disposal exist which are usually performed by businesses specializing in pesticide waste disposal. The technology for pesticide waste disposal is expansive, involving adsorption/landfill (Dennis, 1988), incineration (Bridges and Dempsey, 1988), photolysis (Peterson et al., 1990), ozonation (Kearney, et al., 1990), hydrolysis (Kennedy et al., 1981), biological degradation (Hall et al., 1981), biologically active adsorption to granular activated carbon (Massey et al., 1992), and combinations of ozonation and biological degradation (Somich, 1990). Another alternative disposal method involves bioreactors based on aerobic composting techniques (Snell Environmental Group, 1982; Mullins et al., 1989, 1992; California Agricultural Research Inc., 1992; Shanks et al., 1992; Berry et al., 1993). The highly biologically active state achieved during a composting situation can greatly accelerate the degradation of waste pesticides.

The test system in these experiments was designed to represent the conditions of the pesticide waste disposal system designed by Mullins et al. (1989; 1992). Lignocellulosic materials, such as peat moss, and steam-exploded wood fibers and peanut hulls, are used in the primary step of the pesticide waste disposal system for isolating the
pesticide phase from pesticide-laden wastewater through sorption processes. The resulting lignocellulosic matrix now containing the pesticide phase is placed into bioreactors which are designed to be highly biologically active to facilitate pesticide degradation. The steam-explosion process exposes cellulose, hemicelluloses, and other structural components, creating a larger surface area for sorption of pesticides and a more readily usable energy source for microbial activity.

Composting involves an aerobic and highly biologically active process by which organic matrices, such as yard waste, vegetable-based refuse, organically-based industrial refuse, and other biodegradable materials are used for metabolic energy. Composting is a dynamic process, involving organic matter degradation and a ecological succession of microbial, algal, protozoan, as well as microinvertebrate populations with varying abilities to degrade and use the organic matter for catabolic and anabolic metabolism. Figure 18 (Project III) represents a generalized temperature profile and ecological succession observed in compost (Polprasert, 1989; data from Project IV). MacGregor et al., (1981) stated that the thermophilic period, specifically 52 to 60 °C, is the most conducive period for the degradation of organic matter. The middle 40 to 50 °C range has been reported to be the favorable range for the growth and maintenance of thermophilic microorganisms (Dye, 1964; Miller, 1993). Golueke (1972) reports that most researchers believe that optimal conditions for composting occur between 35 and 55 °C. The intention in this study was to simulate the conditions during the optimal thermophilic period by maintaining experimental bioreactors between 45 and 50 °C.

It has been demonstrated that amending microbial systems with a secondary carbon source and nutrients can increase the biodegradation rate of recalcitrant compounds (Lu and Tsai, 1993; Felsot and Dzanto, 1995). Mullins et al. (1989) demonstrated that amending peat moss based compost with ground corn meal and nitrogen fertilizer increased the rate of diazinon degradation. It was the purpose of this study to determine if nutrient amendment to biobased bioreactors undergoing solid-state fermentation increased pesticide dissipation by enhancing microbial activity. Overall microbial activity can be delineated by observing some metabolic process, such as respiration or adenosine triphosphate (ATP) concentration. In this study, manometric methods for quantitating metabolic oxygen consumption employing a modified manometer (Figure 30) as described by Lein et al., (1972) were used to determine the correlation between overall microbial activity in either nutrient-amended or unamended bioreactors and the degree of pesticide dissipation.
Figure 30  Schematic of Manometer and Bioreactor Apparatus
4.5.3 Materials and Methods

Chemicals
Ciba Geigy Corporation (Greensboro, NC) supplied formulated atrazine as AATrex® 4L, and the following analytical-grade reference standards (purity > 98%): 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-s-triazine [atrazine or ATZ], 2-chloro-4-amino-6-isopropylamino-1,3,5-s-triazine [de-ethylatrazine or DEA], 2-chloro-4-ethylamino-6-amino-1,3,5-s-triazine [de-isopropylatrazine or DIA], 2-chloro-4,6-diamino-1,3,5-s-triazine [didealkyl-atrazine or DAA], 2-hydroxy-4-ethylamino-6-isopropylamino-1,3,5-s-triazine [hydroxyatrazine or HYA], 2-hydroxy-4-amino-6-isopropylamino-1,3,5-s-triazine [de-ethyl-hydroxyatrazine or DEHYA], 2-hydroxy-4-ethylamino-6-amino-1,3,5-s-triazine [de-isopropyl-hydroxyatrazine or DIHYA].

DowElanco (Indianapolis, IN) supplied formulated chlorpyrifos as Dursban® 4E and the following analytical-grade reference standards (purity > 98%): O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate [chlorpyrifos], and 3,5,6-trichloro-2-pyridinol [TCP].

Unless otherwise specified, chemicals were reagent grade and solvents used were at least of HPLC-grade; these were purchased primarily from Fisher Scientific (Atlanta, GA).

Test System
The test system used in this study involved 1/2-pint (237 mL) Mason® jars containing approximately 25 g (dry weight) of a 1:1:1 mixture (w/w; dry weight) of peat moss, steam-exploded wood (SEW), and steam-exploded peanut hulls (SEPH) as the composting matrix. The steam-explosion products were supplied by the Virginia Tech Biobased Materials Center (Blacksburg, VA). The steam-explosion process described by Overend and Chornet (1987) involved a Masonite® process gun. The SEW and SEPH originated from yellow poplar (Liriodendron tulipifera L.) and the SEPH originated from peanut hulls supplied by Birdsong Inc. (Petersburg, VA).

To supply a microbial inoculum to all bioreactors, a bag of Brown Leaf Compost Maker® supplied by Ringer® (Eden Prairie, MN) was mixed into approximately 5 kg of the matrix mixture. Research has shown that the process of vegetable material degradation and anthropogenic compounds is increased or enhanced by adding a microbial
inoculum of appropriate type and size (Golueke and Diaz, 1989; Ramadan, 1990). Treatments to the bioreactors were applications of either formulated atrazine (AAtrex® 4L), chlorpyrifos (Dursban® 4E) at approximately 5000 μg/g dry matrix (ppm), or no pesticide treatment. Additionally, treatments included nutrient amendment or no amendment, where nutrient amendment included an aliquot of a nitrogen, phosphorus, and potassium (NPK) fertilizer (16 mg nitrogen/g dry matrix) with either 160 mg ground corn meal/g dry matrix or 20 mg vegetable oil/g dry matrix. Each individual treatment included 16 replicates, 4 replicates each to be sacrificed for extraction at 0, 4, 8, and 16 weeks after initiation of incubation.

The matrix in each bioreactor was mixed well with the proper amendment, and then a saturating amount of distilled H₂O was added to the point where H₂O began to puddle in the bottom of the bioreactor. Each bioreactor (1/2 pint (473 mL) Mason® jar) was covered with waxpaper and two to three pin-holes (< 0.5 mm) were made for adequate gas exchange, while minimizing the loss of H₂O evaporation and pesticide volatilization. It was determined by gas chromatography (Methods in Project III) in a preliminary experiment that the oxygen concentration in the waxpaper-covered bioreactor was the same as ambient oxygen.

Temperature and Experimental Management

Bioreactors were kept at 45 ± 5 °C in walk-in chamber constructed especially for maintaining higher temperatures (Figure 31). The chamber was heated and the heat regulated by a household space heater (circulating-oil type). Room temperatures were collected once an hour with a Campbell Instrument (Denver, CO) datalogger and type-T thermistor (Omega Instrument). On warmer days the temperature in the insulated room would approach 50 °C and then cool to near 45 °C as the outside temperatures dropped at night.

Additional amendments of either corn meal + fertilizer (N, P, K) or vegetable oil + fertilizer occurred on days 29, 51, and 96 after the initial application of the pesticides and nutrients. The matrix in all bioreactors was rehydrated with tap water to the point just beyond saturation, and the contents mixed well on days 18, 45, 77, and 106 after experiment initiation. Homogeneity was ensured by mixing the matrices during all nutrient and water amendments.
Figure 31: Schematic of Insulated Chamber
Manometry Procedures

Figure 30 represents the device used during this study constructed for manometric determinations of oxygen consumption based on the design of Klein et al. (1972). Each side of the U-tube was 61 cm tall constructed from glass tubing (0.3175 cm internal diameter or i.d.) connected with 15 cm of Tygon® tubing (0.3175 cm i.d.). Attached between the manometer jar and one side of the U-tube was 30.5 cm of Tygon® tubing (0.3175 cm i.d.). The manometer jar used was a 1 quart (946 mL) Mason® jar with an inlet and outlet tubes in the cap made from 1 mL plastic syringes (B&D, Princeton, NJ). Silicone caulking was placed around the syringes where they penetrated the caps to prevent leaking. The manometer unit was constructed from 1 by 6 inch pine lumber and the base and vertical stand were both 45.7 cm long. Metric rulers were placed behind the U-tubes to designate the level of the manometer changes during the manometry reading. For manometric readings of pressure changes, 1.0 mL aliquots of Kreb's manometer fluid (Umbreit, et al., 1964) was put into each U-tube. Kreb's manometer fluid was made in 1 L of distilled H₂O by dissolving 44 g NaBr and 0.3 g Triton X-100 (Rohm & Haas, Co.).

The manometric procedures used during this study were similar to those described by Klein et al. (1972). Random triplicate representatives of each treatment were taken at varying time intervals and placed into the manometers' 1 quart Mason® jars. Three manometers were designated as thermobarometers which functioned as a control for recording the changes of pressure that were due to chamber temperature and atmospheric pressure fluctuations. A 7.5 cm dia. plastic mesh (0.5 x 0.5 cm) screen was placed on top of all 1/2 pint jars inside the 1 quart manometer jars. A 40 mL glass jar containing 10 mL of a 5M KOH solution was placed on the mesh screen, which absorbed the CO₂ evolved from the bioreactors. The manometric jar containing a bioreactor was closed tightly and attached to the U-tube (Figure 30) of the manometric unit. The relief valve on the quart jar was closed for 24 hours to equilibrate during a refractory period. After 24 hours, the relief valve was loosened, allowing the internal pressure to equilibrate with atmospheric pressure. The relief valves were then closed, and the temperature, time, and manometric solution levels for each manometer were recorded. It was determined in preliminary experiments that due to oxygen consumption rates and the sensitivity of the manometers, 30 minutes was the best time interval for reading the manometer. Thirty minutes after initiation, the temperature, time, and level of the manometer solution were recorded. Afterwards, the bioreactors were disconnected from the manometers.
The consumption of oxygen in each manometer per reading was calculated using the following equation:

\[
V_{O2} = h \times k;
\]
where \( V_{O2} \) is the volume of oxygen consumed in mL
\( h \) is the actual pressure change (mm) over the reading interval
\( [h = \text{change in mm - change in mm of the thermobarometer}] \)
\( k \) is the manometer constant (mL) \{see equation 2\}

\[
k = \left( V_g \times \frac{273}{T} \right) + \left( V_f \times \alpha \right)
\]
\( P_0 \)
where \( k \) is the manometer constant in mL
\( V_g \) is the void volume of the manometer in \( \mu \)L (7.51 \times 10^5 \( \mu \)L)
\( V_f \) is the aqueous volume in the manometer in \( \mu \)L (1.0 \times 10^4 \( \mu \)L)
\( T \) is the temperature in \( ^\circ K \) (300 \( ^\circ K \))
\( \alpha \) is the oxygen absorption coefficient \( (2.3 \times 10^{-3} @ 40 \, ^\circ C) \)
\( P_0 \) is the standard pressure of Kreb's fluid \( (1.0 \times 10^4 \, \text{mm}) \)

The manometry flask constant was determined to be \( 64.5 \pm 6.3 \) (± sem) for all flasks. The variability between the volume of the gas in the manometers (\( V_g \)) was minimal, varying only with difference between the volume of corn meal vs. vegetable oil added, therefore, the value of 64.5 was used to calculate the change in the volume of oxygen per time unit for all flasks.

Matrix Sampling and Extraction

Four replicates of each treatment were removed for extraction and analysis the day of application and at 4, 8, and 16 weeks after experiment initiation. The contents of each bioreactor were mixed well by hand and the weight of matrix recorded. The ratio of wet vs. dry matrix from each bioreactor was determined by transferring approximately 10 g of the wet matrix into 1.0 g aluminum weighing dishes, recording the wet matrix weight,
drying the matrix at 110 °C for 48 hours, and recording the dry matrix weight. Based on
the wet/dry ratio, weighed proportions of the wet matrix approximating 1.0 g dry matrix
were transferred to 40 mL polypropylene centrifuge tube (Fisher Scientific, Greensboro,
NC). Twenty mL of ethyl acetate was added to each centrifuge tube containing the
matrix, the contents shaken by hand for one minute, then allowed to sit overnight, not
exceeding 12 hours. The centrifuge tubes were then placed into a sonicating water bath
(Fisher Scientific) for three separate 30 minute periods with 30 minute intervals of not
sonicating. The contents were centrifuged at approximately 3000 rpm (1000 g; Beckman
Centrifuge, Beckman Instrument, Inc., Irvine, CA) for 15 minutes. The supernatant was
decanted into 500 mL beakers, which were placed in a fume hood to reduce the volume.
The extraction process was repeated for a total of four times, repeating the addition of 20
mL of ethyl acetate, the sonication process, the centrifugation, and decanting the
supernatant into the same 500 mL beaker. After the fourth extraction the matrix was
allowed to dry before being stored at -20 °C. The volume of the collective supernatant
from each bioreactor was reduced to approximately 45 mL in a fume hood and then
adjusted to 50 mL with ethyl acetate. Twenty mL aliquots were taken and stored at -20 °
C and an additional 4 mL aliquot was taken for chromatographic analysis.

Previous recovery experiments or extraction efficiency experiment involving
spiking small amounts of matrix (2 g dry) with 1 mg atrazine and chlorpyrifos standards
were quite variable and dependent on the amount of time allowed before extraction.
Atrazine recoveries using the above extraction procedures were 72 ± 9% (mean ± standard
error of the mean, sem), ranging from 52 to 87%. The 52% recovery resulted from
samples allowed to sit at room temperature for 48 hours before extraction. Chlorpyrifos
recoveries were 94 ± 3% of the chlorpyrifos originally applied, ranging between 92 to
107%. The 48 hour sitting time did not appear to affect the recovery as it did with
atrazine. The extraction efficiencies of the other metabolites were not determined. The
extraction efficiencies of hydroxyatrazine and TCP were good, considering that these two
metabolites were found in high concentration. It is not obvious how efficient the ethyl
acetate extraction was for the more water soluble atrazine metabolites. Ethyl acetate is
fairly non-polar and the more water soluble atrazine metabolites (DAA, DIA, DEA,
DIHYA, DEHYA) may not have been extracted well from the matrix. Therefore, the
more water soluble metabolites recovered (Figures 37 and 38) may have actually been in
the matrix in larger amounts. It is possible that the ethyl acetate was efficient in extracting
the more water soluble atrazine metabolites, since Kruger et al. (1993) was able to
partition all of the atrazine metabolites used here from a 9:1 methanol:water solution to dichloromethane.

**pH of Matrix**

The pH of each bioreactor was determined at the time the sample was taken for extraction. A portion of the wet matrix (0.8 x wet wt./dry wt.) from each bioreactor was transferred to a 50 mL beaker, 20 mL of distilled H₂O was added, and the contents were mixed with a magnetic stir bar for 30 minutes. See the Methods Section 4.3.2.2 in Section III for more detail on why the pH formula (0.8 x wet wt./dry wt.) was used to calculate pH. The pH was then recorded after 15 minutes of immersing a combination pH electrode with silver/silver chloride references (Fisher Scientific) in an non-mixing matrix slurry.

**High Performance Liquid Chromatography (HPLC)**

High performance liquid chromatography (HPLC) was performed with all samples using a Hewlett Packard HPLC unit (Model No. 1050; Hewlett Packard Co., Avondale, PA) with a low pressure solvent-mixing unit, a Rheodyne (Coati, CA) Model 7125 injection valve fitted with a stainless-steel 20 μL injection loop, and a ultraviolet-visible (UV-VIS) detector (Hewlet-Packard Model no. 1050). The chromatographic column used for all analyses was a Nucleosil® C₁₈-HS-bonded silica column (250 mm long by 4.6 mm in internal diameter, 5 μm particle-size, 60 Å pore-size, 18% w/w carbon load, endcapped silanols; Alltech). All solvents were continuously sparged with helium, and flow rates were maintained at 1.0 mL/minute.

Chromatograms were displayed on a Hewlett Packard printer/integrator (Model 3396). Peaks in the chromatograph were identified by retention time (Rt) comparison to retention times of known standards. In certain circumstances, confirmation of peak identity was done by spiking the same sample with a standard and then repeating the chromatography. Quantitation of analyte amounts was done by extrapolating the UV response of the analyte to a standard curve of the particular compound being quantitated. Standards of atrazine, chlorpyrifos, and their metabolites in ethyl acetate between 0.005 and 1000 μg/g were used to construct a standard curve. A standard curve consisted of at least three different amounts of the standard and coefficients of determination (r²) were greater than 0.94.
Atrazine Chromatographic System

The chromatographic analysis of the atrazine samples was based on the method described by Wenheg et al. (1991) and performed under reverse-phase conditions using an ion-pairing water/acetonitrile ternary gradient at a fixed wavelength of 225 nm. The solvent reservoirs consisted of a H$_2$O, acetonitrile (ACN), and a 50/50 H$_2$O/ACN reservoir. The H$_2$O and 50/50 H$_2$O/ACN reservoirs were 0.020 M n-heptanesulfonic acid (Kodak, Hartford, CN) and 0.10 M H$_3$PO$_4$. The following Table 19 represents the gradient:

Table 19  HPLC Gradient for Separating Atrazine and Several of its Metabolites

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>% H$_2$O Reservoir</th>
<th>% ACN Reservoir</th>
<th>50/50 H$_2$O/ACN Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>37</td>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

1 ACN is acetonitrile

The retention times in minutes of atrazine and metabolite standards were 4.5 (DAA), 7.1 (DIHYA), 8.5 (DEHYA), 9.1 (DIA), 12.5 (DEA), 13.4 (HYA), and 21.7 minutes (ATZ). Based on the sensitivity of the UV-VIS detector for standards of atrazine at a wavelength of 225 nm and the volume of the extract, the limit of determination (LOD) for atrazine was 0.05 µg/g dry matrix (ppm). The LOD for the metabolites were as follows: DAA (0.05 µg/g), DIHYA (0.1 µg/g), DEHYA (0.05 µg/g), DIA (0.15 µg/g), DEA (0.1 µg/g), HYA (0.05 µg/g)

Chlorpyrifos Chromatographic System

The chromatographic analysis of the chlorpyrifos samples using a modified method described by Racke et al. (1990), which was performed under reverse-phase conditions using a water/methanol binary gradient at a fixed wavelength of 300 nm. Both solvent
reservoirs were 0.05% (w/w) N,N-diethylloctylamine (Sigma Chemical, Chicago, IL) and 1% (v/v) acetic acid. The gradient used was as follows:

Table 20  HPLC Gradient for Chlorpyrifos and 3,5,6-trichloropyridinol

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Percent of Total Flow (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H₂O Reservoir</td>
</tr>
<tr>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

¹ACN is acetonitrile

The retention times for standards of chlorpyrifos and TCP were approximately 11.4 and 6.4 minutes. Based on the sensitivity of the UV-VIS detector for chlorpyrifos and TCP at a wavelength of 300 nm and the volume of the extract, the LOD for both chlorpyrifos and TCP in this study was 5 μg/g dry matrix (μg/g).

Statistics

Where appropriate, data will be presented as a mean ± the standard error of the mean (sem). To determine if significant differences (α = 0.05) were present between certain treatments, either Student's t-test or an analysis of variance (ANOVA) was performed. The ANOVA was performed using the general linear method (GLM) procedure in the computer-based Statistical Analysis Systems (SAS Institute, 1982).

Calculations of DT₅₀ values or half lives were calculated using pseudo-first order rate kinetics and the concentrations of the parent pesticide recovered from the solvent-extractable residue using HPLC. The following equation represents the DT₅₀ calculation:

\[
DT₅₀ = \frac{\ln 2}{k}
\]

where the DT₅₀ is the time it takes 50% of the original amount of pesticide to dissipate, and the "k" is the DT₅₀ coefficient unique to each pesticide in each situation. The DT₅₀ coefficient (k) is the slope of line from a plot of the ln of the pesticide concentrations or percentages vs. their respective times on the abscissa.
4.5.4 Results and Discussion

Matrix pH

Table 21 presents the average pH determined for each treatment at the experiment initiation and at 4, 8, and 16 weeks. The initial matrix pH values before pesticide application were neutral at pH values between 7.2 and 7.7. Over the 16 week period, the pH in all bioreactors remained between 6.0 and 8.3. After 16 weeks, the lowest pH values were observed in all bioreactors receiving no nutrients (6.5 to 6.8). The highest pH observed was 8.3 at 8 weeks in the corn meal amended bioreactors.

According to Polprasert (1989), as long as the composting process remains aerobic, the pH usually does not vary much over time. Mathur et al. (1990) reported a slight increase of pH in a peat moss-based compost after one week at 65 °C. After 16 weeks, the pH values in the vegetable oil amended and bioreactors not amended for both atrazine and chlorpyrifos were slightly more acidic than at the beginning of the experiment (Table 21). However, the pH increased slightly in the corn meal bioreactors for both atrazine and chlorpyrifos.

Microbial Activity

Figures 32 and 33 compare the microbial activities in the bioreactors represented as the oxygen consumption rates (µL/minute) for atrazine and chlorpyrifos bioreactors. Figures 34 and 35 represent the same data presented in Figures 32 and 33, but in a cumulative presentation rather than an oxygen consumption rate (µL/minute). Oxygen consumption data in the form of rates are important for graphically examining bioactivity responses after nutrient amendment. The same data presented in a cumulative fashion are better for comparing the total effect on bioactivity by nutrient amendment and allows or statistical analysis. Cumulative oxygen consumed per treatment could not be calculated accurately due to changing rates between measurements. However, cumulative oxygen consumption rates for each treatment are proportional to the amount of oxygen that was consumed for each treatment.

In both the atrazine and chlorpyrifos bioreactors, the initial microbial activity in the corn meal amended (CMA) and vegetable oil amended (VOA) bioreactors was immediately greater than in the bioreactors not amended (NA). During the next three
<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-Time</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>16 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atrazine</td>
<td>Chlorpyrifos</td>
<td>Penicilide</td>
<td>Atrazine</td>
</tr>
<tr>
<td>Vegetable Oil Amended</td>
<td>7.4</td>
<td>7.1</td>
<td>6.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Corn Meal Amended</td>
<td>6.0</td>
<td>6.9</td>
<td>6.9</td>
<td>7.7</td>
</tr>
<tr>
<td>Not Amended</td>
<td>7.2</td>
<td>7.7</td>
<td>6.9</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Matrix: 35 g (dry weight basis) of a 1:1 mixture of steam-exploded wood, peat moss, steam-exploded peanut hull

Vegetable Oil Amended: rate of 20 mg vegetable oil/g dry matrix

Corn Meal Amended: rate of 160 mg corn meal/g dry matrix

Atrazine: initially 4759 +/- 499 mg atrazine/g dry matrix (delivered as AATv 4L)

Chlorpyrifos: initially 450 +/- 274 mg chlorpyrifos/g dry matrix (delivered as Deltan 4F)

sem = standard error of the mean (n = 3)
Figure 32 Oxygen Consumption Rates for Atrazine Bioreactors
Figure 33 Oxygen Consumption Rates for Chlorpyrifos Bioreactors
Figure 34  Atrazine Bioreactors: Cumulative Oxygen Consumption Rates (μL/minute)

Vertical bars represent the standard error of the mean
Figure 35  Chlorpyrifos Bioreactors:  Cumulative Oxygen Consumption Rates (µL/minute)

Vertical bars represent the standard error of the mean
weeks, the microbial activity increased slightly in both atrazine and chlorpyrifos bioreactors amended with vegetable oil, but decreased slightly in CMA bioreactors containing either atrazine or chlorpyrifos. Amending the bioreactor matrices with nitrogen, phosphorus, and potassium (NPK) fertilizers along with either corn meal or vegetable oil significantly (P = 0.002) increased microbial activity as indicated by the temporal and cumulative oxygen consumption rates over the duration of the experiment.

During the course of the 16 week atrazine experiment, it appears that microbial activity patterns were similar among bioreactors treated with the same nutrient amendment (e.g. the microbial activity in VOA bioreactors of atrazine vs. no pesticide; Figure 32). Total cumulative microbial activity in the atrazine bioreactors over the entire 16 week incubation, denoted by cumulative oxygen consumption rates in Figure 34 was significantly enhanced by amending bioreactors with corn meal or vegetable oil (P = 0.001; ANOVA). ANOVA performed on the class variables, pesticide and feeding regime, resulted in no significant differences of cumulative oxygen consumption rates between VOA and CMA bioreactors (P = 0.72). Corn meal was added at amounts eight times greater than the weight of vegetable oil with the ideal to equalize the amount of actual metabolic energy available for microbial metabolism. In general, carbohydrates have a respiratory quotient (RQ) or energy from oxidation of 1.00, and lipids have been assigned RQ's of less than 1.00 (Battley, 1989). Thus, a lesser amount of lipid compared to carbohydrates can be used metabolically for approximately the same energy. The factor that vegetable oil or lipids will yield eight times the metabolic energy over the same weight of the corn meal (carbohydrate) was taken from a reference in insect flight (Beenakkers et al., 1986), and substantiated in an article by Mathur (1991).

In all bioreactors, a peak of microbial activity occurred on day 25 after the first mixing event which occurred on day 18 (Figures 32 and 33). It is common management practice to mix the matrix in composting situations to redistribute nutrients and populations of microorganisms (Biddlestone et al., 1987). Mixing allows new sites and unused nutrients for growth to become available to redistributed microorganisms.

There was a second round of heightened microbial activity on day 37 after reapplying nutrients to the bioreactors on day 29. Over the following approximately 2 weeks, there was a decrease observed in all bioreactors. On day 45 the matrices in all bioreactors were mixed and rehydrated. Because only 6 days passed from mixing and reapplication of nutrients, it is difficult to conclude that the mixing event would not have stimulated microbial activity as it had in past mixing events.
The largest spike of microbial activity over the course of the experiment in all bioreactors occurred on day 57, six days after the nutrients were applied on day 51 (Figures 32 and 33). On day 57 there were no significant differences (P > 0.1) of oxygen consumption rates between CMA or VOA bioreactors in either pesticide treatment. It appears from Figures 32 and 33 that the microbial oxygen consumption rates were higher in the CMA bioreactors not containing either atrazine or chlorpyrifos compared to their atrazine or chlorpyrifos-containing counterparts. However, there were no significant differences between oxygen consumption rates on day 57 in both atrazine-treated or untreated bioreactors (VOA bioreactors, P = 0.96; and CMA bioreactors, P = 0.46) (Figure 32). Similarly, there were no significant differences in oxygen consumption rates observed between chlorpyrifos-treated bioreactors versus bioreactors not treated (VOA bioreactors, P = 0.23; and corn meal amended bioreactors, P = 0.41) (Figure 33). Matrix mixing occurred on day 77, and followed the characteristic microbial activity peaks. On day 96, nutrients were reapplied to the appropriate bioreactors, but unlike the preceding periods following nutrient reapplication, only a small increase of microbial activity was subsequently observed. The observed spikes of microbial activity after amendment or mixing events appeared to be more pronounced early in the experiment. Towards the end of the experiment, mixing events or the addition of nutrients on day 96 did not appear to create the same magnitude of microbial activity within each treatment (Figures 32 and 33).

The decrease in microbial activity observed at the end of the experiment would suggest that there was some toxicity to microorganisms occurring. However, because the non-atrazine or non-chlorpyrifos containing bioreactors had similar decreases in microbial activity, the inferred toxicity must have been due to something other than a direct toxic effect from the pesticides. The overall decrease in microbial activity observed in all of the bioreactors could have occurred due to a combination of certain species out competing others, the presence of antimicrobial compounds produced by a predominating species or species of microbes, and a subsequent phase of inactivity, be it senescence, sporulation, or metabolic crash after a fruiting period. It is common for microbial populations to produce antibiotics with the sole purpose to reduce competing species through toxicity (Ross and Harris, 1983; Kai et al., 1990).

The other possibility involves microbial ecology and energy supply available. It has been shown that fungi and/or actinomycetes will become the predominate species in a composting situation though normal succession (Polprasert, 1989; Miller, 1993). White mycelia mats in certain bioreactors were observed, which may have been species of fungus
or actinomycetes. It has been shown in previous research (Project IV) that fungi and actinomycetes did predominate in compost late in the succession. The microbial populations present in all bioreactors could have been experiencing a death or senescence phase. A microbial population decline may have occurred due to the depletion of nutrient resources, considering that there was a much longer period between nutrient amendments occurring at day 51 and then day 91. Approximately 40 days had elapsed since the last nutrient amendment on day 51 until the amendment on day 91. The experiment was completed 24 days after the nutrient amendment on day 91, which is similar to the lag time observed at the onset of the experiment before a spike of activity occurred. It is likely that as the readily available nutrient source was depleted, the microbial populations present produced spores or fruiting bodies and then entered a dormant phase. It is possible that if the incubation period had been extended, the apparent lag or senescence phase might have been followed by a measurable increase in microbial activity.

Initially, it appears in Figure 34, a that the addition of atrazine of chlorpyrifos may have resulted in reduction of the overall microbial activity, except in the VOA bioreactors containing chlorpyrifos where microbial activity appeared to be enhanced. However, there were no significant pesticide effects on the final cumulative oxygen consumption rates between any of the atrazine or chlorpyrifos nutrient-amended bioreactors (P = 0.72; ANOVA). A Student's t-test did indicate that there was significant inhibition (P = 0.047) of microbial activity in CMA bioreactors containing atrazine to those CMA bioreactors without atrazine. Table 22 summarizes the results from comparisonwise Student's t-tests between pesticide treated and not treated groups.

Table 22  Significance of Pesticide Effects on Cumulative Oxygen Consumption Rates

<p>| Cumulative Oxygen Consumption Rates are Means +/- Standard Error of the Mean in Units of µL/minute |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Vegetable Oil</th>
<th>Corn Meal</th>
<th>Not Amended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine (9824 ± 323) vs. No Atrazine (10504 ± 491) P = 0.34</td>
<td>Atrazine (8027 ± 639) vs. No Atrazine (125967 ± 1456) P &gt; 0.047</td>
<td>Atrazine (2385 ± 566) vs. No Atrazine (2303 ± 504) P = 0.95</td>
</tr>
<tr>
<td>Chlorpyrifos (11554 ± 772) vs. No Chlorpyrifos (10504 ± 491) P = 0.31</td>
<td>Chlorpyrifos (9241 ± 815) vs. No Chlorpyrifos (125967 ± 1456) P = 0.11</td>
<td>Chlorpyrifos (2461 ± 486) vs. No Chlorpyrifos (2303 ± 504) P = 0.97</td>
</tr>
</tbody>
</table>

1Cumulative oxygen consumption rates used were the totalled cumulative oxygen consumption rates for each treatment, which are proportional to total oxygen consumption over the entire 106 day incubation
There is variable information available in the literature regarding pesticides that have inhibitory or stimulatory effects on the microbial activity. It is difficult to conclude that the pesticides in a soil environment are toxic to microorganisms. In this experiment, the presence of atrazine in compost significantly decreased overall microbial activity (P = 0.047) over the 16 week experiment in CMA bioreactors (Figure 34). For the most part, atrazine has not been implicated in inhibiting soil microbial activity at normal field rates of approximately 3.4 kg/ha (Wagner and Chahal, 1966; Wolf and Martin, 1975; Cole, 1976). Atrazine in soil has been shown to have stimulatory effects on microbial growth and activity at normal applicate rates, as well as exaggerated rates (Tu, 1992). Simazine, another triazine herbicide related to atrazine, did not affect the degradation and mineralization of straw in soil (Harden et al., 1993). The majority of studies determining the affects of atrazine on soil microbial activity were based on atrazine concentrations near field rates (3.4 kg/ha), but in this study atrazine was applied to compost at rates many times greater. It may be that atrazine or its metabolites may be toxic to microorganisms at the elevated concentrations of approximately 5000 μg/g. Also, because atrazine was applied at such high rates as AAtrex® 4L, it may have been that one or more of the carrier/inert chemicals in the formulations were inhibitory to microbial activity.

It is interesting that adding chlorpyrifos at approximately 5000 μg/g did not significantly affect microbial activity. Felsot and Pedersen (1991) reported that 3,5,6-trichloro-2-pyridinol (TCP) inhibits the growth of several species of fungi in radial growth toxicity tests. It has been shown that TCP, the main metabolite of chlorpyrifos, can adversely affect or inhibit microbial activity in soil (Sivasithamparam, 1970; Wood and McRae, 1974). They suggest that as TCP accumulated in soil due to the hydrolysis of chlorpyrifos, cellular respiration was disrupted and reached a toxic level for certain microbial activity. The formation of TCP did occur in this study and after the first 4 weeks of the experiment, TCP concentrations remained between 500 and 2500 μg/g for the remainder of the experiment. In fact, the presence of chlorpyrifos formulated as Dursban® 4E actually appeared to stimulate microbial activity in the corn meal bioreactors on days 57 and 83 (Figure 33). In another study, Tu (1980) observed that microbial activity was stimulated in soil treated with chlorpyrifos at 5 μg/g.

Some researchers have suggested that stimulation of microbial activity may actually be what is called a "sub-toxic stress effect on certain microbial populations (Killham, 1985). This phenomenon is thought to occur in carbon-limited soil environments, which is the case for most soil ecosystems. In a carbon-limited
environment, a toxic effect on certain populations of microbial species may cause them to shunt their largely anabolic metabolism to strictly maintenance metabolism or catabolism, which would be observed as increased oxygen consumption. Also, a stimulatory effect may be observed when certain microbial populations are able to ecologically outcompete those microbial populations adversely affected by having a toxin present. The addition of pesticides do represent a new and possibly easily metabolized carbon source in an otherwise carbon-limited environment. Testing for pesticide toxicity on microbial activity is best done when carbon is in good supply, and not limited to avoid confounding results from a "subtoxic effect" (Gerber et al., 1989). The composting situation studied here is far from being the carbon-limited environment found in soil. In fact, there was plenty of organic material in the bioreactors compared to soil, therefore, it can be concluded that any significant inhibition are a toxic response.

The addition of NPK fertilizer and either corn meal or vegetable oil markedly increased the overall cumulative microbial activity over the 16 week study (Figures 34 and 35). The temperature was maintained between 45 and 50 °C, which increased overall metabolism and approached the optimal temperature region for those microbial populations that best decompose vegetative material (MacGregor, 1981). Because there was a good carbon supply in the bioreactors, it can be correctly concluded that the addition of atrazine as AAtrex® 4L at high initial concentrations of approximately 5000 μg/g did significantly inhibit microbial activity in CMA bioreactors.

As the results indicate, the microbial ecology was affected by the presence of atrazine, chlorpyrifos, their metabolites, or the various formulation chemicals added to the bioreactors. The two kinds of nutrients were chosen to compare differences in pesticide dissipation brought about by the possibly different predominant microbial consortia in the CMA and VOA bioreactors.

**Pesticide Dissipation**

Information was obtained regarding the concentrations of atrazine and chlorpyrifos and some of their common metabolites. The results obtained by the treatments will now be discussed.

**Atrazine Dissipation**

The addition of nutrients in this experimental series did not significantly increase atrazine dissipation (Figure 36). After 16 weeks at approximately 45 °C, the
Figure 36: Concentration of Atrazine and OH-Atrazine in Compost over 16 Weeks

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>0-Time</th>
<th>Vegetable Oil</th>
<th>Com Meal</th>
<th>Not Amended</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatment (amendment to bioreactor):

- **0-Time**
- **Vegetable Oil**
- **Com Meal**
- **Not Amended**

20 mg/g dry matrix
160 mg/g dry matrix

Amended bioreactors also received 16 mg soluble nitrogen/g dry matrix

Vertical bars represent standard error of the mean.
The same letters at each time point designate significant similarity (alpha = 0.05).
concentration of atrazine declined to approximately 0.3% of the initial \(4759 \pm 419\ \mu g/g\) for all treatments (Figure 36). The DT\(_{50}\) values for atrazine dissipation were calculated to be approximately 0.7 weeks (\(r^2 = 0.96\)) in corn meal amended (CMA) bioreactors, 1.0 weeks (\(r^2 = 0.96\)) in vegetable oil amended (VOA) bioreactors, and 1.1 weeks (\(r^2 = 0.96\)) in the bioreactors not amended (NA). Even though having corn meal present did appear to increase atrazine dissipation at 4 weeks, there were no significant dissipation differences after 16 weeks in the CMA, VOA, and NA bioreactors.

After 4 weeks, amendment with corn meal did significantly increase atrazine dissipation (Figure 36) compared to bioreactors with no nutrients added (\(P = 0.006\)). After 8 weeks, the atrazine concentration of the CMA bioreactors (5.4 ± 3.9 \(\mu g/g\)) and NA bioreactors (16.4 ± 5.4 \(\mu g/g\)) were significantly similar (\(P = 0.15\)). The VOA bioreactors with 36.7 ± 2.8 \(\mu g/g\) atrazine were significantly greater than either the CMA bioreactors (\(P = 0.002\)) or NA bioreactors (\(P = 0.03\)). After 16 weeks, any effects on atrazine dissipation by amending bioreactors with nutrients were not significant (CMA vs. NA, \(P = 0.99\); VOA vs. NA, \(P = 0.44\); and CMA vs. VOA, \(P = 0.44\)).

Atrazine is not considered to be a volatile compound with a vapor pressure of 0.037 mPa (Howard, 1991). Howard (1991) indicates that little volatilization would occur even with temperatures between 45 and 50 °C. Results from Project VI also indicate that atrazine does not volatilize to any great extent. Less than 1% of the totally applied \(^{14}\)C-atrazine was recovered as volatiles after 16 weeks incubation in a compost bioreactor similar to one studied here. Therefore, volatilization of atrazine or its metabolites was ruled out as a path of dissipation.

The additional 8 weeks incubation time between the 8 and 16 week time points did not appear to facilitate further atrazine dissipation (Figure 36). In the CMA, VOA, and NA bioreactors, the atrazine concentration had declined after 16 weeks to 14.8 ± 6.2 \(\mu g/g\), 10.8 ± 4.7 \(\mu g/g\), and 9.7 ± 5 \(\mu g/g\), respectively, or approximately 0.2% of the initial atrazine concentration of approximately 4933 \(\mu g/g\) (Figure 36).

There was approximately 17 ± 14 \(\mu g/g\) of HYA found in the matrix of all bioreactors at the experiment initiation, which was likely formed during the 2 hour period after it was applied to matrix before the samples were frozen (-20 °C). Less than 1 \(\mu g/g\) of HYA was recovered from spiked matrix that was extracted after 2 hours and then analyzed, therefore, it appears that atrazine degradation to HYA occurred after application. Some atrazine hydrolysis may have occurred during storage at -20 °C or during the thawing period, but it is unlikely that the hydrolysis accounted for the amount
of HYA observed after 4, 8, and 16 weeks (Figure 36). Armstrong and Chesters (1968) demonstrated that atrazine was hydrolyzed to HYA through a sorption-mediated process. As hydrogen bonding occurs between the triazine ring nitrogens and matrix carboxylic functional groups (Gamble and Khan, 1985), the electron deficiency of the triazine ring carbons is increased making the hydrolysis of the chlorine more susceptible for hydrolysis. It was likely that sorption-mediated hydrolysis occurred as the formulated atrazine was in contact with matrix for approximately 2 hours before samples were frozen.

The primary degradative pathway for atrazine in this test system was by hydrolysis to HYA due to relatively large amounts of HYA detected at all time intervals sampled. As the experiment proceeded, the HYA concentration continually increased in all treatments over the 16 week period (Figure 36). In the VOA bioreactors, the HYA concentration increased from $479 \pm 15$ µg/g after 4 weeks to $597 \pm 66$ and $616 \pm 55$ µg/g after 8 and 16 weeks. The HYA concentration increased in the CMA bioreactors from $359 \pm 20$ µg/g after 4 weeks to $547 \pm 60$ and $788 \pm 75$ µg/g after 8 and 16 weeks. In the NA bioreactors, the HYA concentration increased from $475 \pm 37$ µg/g after 4 weeks to $666 \pm 25$ and $749 \pm 66$ µg/g after 8 and 16 weeks. Only at the 4 week time point was the HYA concentration significantly different in one treatment. The concentration of HYA of $359 \pm 20$ µg/g in the CMA bioreactors was significantly lower than $479 \pm 14.7$ µg/g or $474.8 \pm 37.1$ µg/g in the VOA and NA bioreactors, respectively ($P = 0.023$).

It is widely accepted that the hydrolysis of atrazine to HYA is the major degradative pathway and is mainly an abiotic process (Skipper et al., 1967; Benyon et al., 1972, Kaufman, 1974; Muir and Baker, 1978; Kahn and Saidak, 1981; Jones et al., 1982; Winkleman and Klaine, 1991a). However, Mandelbaum et al. (1993) have shown that the hydrolytic process can be mediated by microbial metabolism. They suggest that the authors of studies concluding that atrazine hydrolysis occurred similarly in sterilized soil compared to active soils may have overlooked the fact that soil enzymes were not destroyed during the sterilization process. Most sterilization methods are not completely effective, thus surviving microorganisms continue to grow.

By the end of the 16 week experiment the dissipation of atrazine was similar in all treatments. In the NA bioreactors, it was evident microbial activity was much lower than in the nutrient-amended bioreactors (Figures 34 and 35). Mandelbaum et al. (1993) have shown that nutrients, such as ammonium nitrate and sucrose could not be used by those bacterial isolates capable of degrading atrazine. Therefore, it is possible that microbial consortia which were present in the NA bioreactors, having considerably lower overall
activity, could have been capable of using atrazine as a carbon source very effectively. The probability of this occurring may have been even greater if an atrazine-degrading consortium could grow without the matrix being dominated by quickly growing microbial populations capable of metabolizing vegetable oil or corn meal.

At week 4, it appeared that dissipation of atrazine was significantly increased by microbial activity (Figure 36). It is difficult to conclude that the observed significant atrazine dissipation was achieved exclusively by hydrolysis. At the same time (4 weeks) that the atrazine concentration was significantly lower in the CMA bioreactors, the HYA concentration was also significantly lower (Figure 36). It may have been that the microbial consortium and the enzyme complexes present in the CMA bioreactors were both effective in the hydrolysis of atrazine and degrading the HYA as it was formed. Of course, the degradation of atrazine may have resulted in a product other than HYA. The dealkylation of atrazine to de-ethylatrazine or deisopropylatrazine has been shown to occur in soils and be an enzymatically-mediated process (Kaufman and Blake, 1970; Giardina et al., 1980; Behki and Khan, 1986; Winkleman and Klaine, 1991a).

In addition to analyzing for atrazine and its major hydrolytic product HYA, analyses were conducted to determine if the following metabolites were present in the compost matrices: didealkylatrazine (DAA), deisopropylatrazine (DIA) de-ethylatrazine (DEA), deisopropyl-OH-atrazine (DIHYA), and de-ethyl-OH-atrazine (DEHYA). Verification of peaks with retention times the same as the metabolite standards was done by spiking known standards into samples having peaks with retention times of standards. This is not a conclusive test for identification of unknowns in the chromatography, but does give more confidence identifying peaks in the chromatography. Further analyses were not done to determine structural identification (i.e. mass spectrometry).

An analytical problem was encountered during the separation of deisopropylatrazine (DIA) due to a problem in the chromatography with a large interfering peak. The large interference peak originated from the organic matrix used in all bioreactors, since the peak was present in all extracts, including those without atrazine or chlorpyrifos. Adjusting the mobile-phase make-up and flow rate did not achieve a separation of the interfering peak on the C-18 bonded phase HPLC column. Other HPLC columns with different bonded phases were not tried. However, there were some incidences of DIA in the HPLC that were separated from the larger peak and ranged between 0.1 and 1.4 µg/g. Some samples were assayed by gas chromatography for
verification of the parent compounds, and there were a few peaks of DIA present, but only at concentrations below 1 μg/g.

Even though deisopropyl atrazine was not quantitated, DEA, DAA, DIHYA, and DEHYA were found in the samples over the 16 week incubation period (Figures 37 and 38). All of the aforementioned metabolites were also found in samples frozen 2 hours after the application of the parent compound (Figures 37 and 38). The formation of the dealkylated metabolites during the first 2 hours further supports the conclusion that microbial metabolism of atrazine was occurring. It is unlikely that the chemical degradation occurred during the extraction process, because no metabolite residues, except HYA, were detected in samples spiked with the formulated atrazine (AAtrex® 4L) in preliminary recovery experiments after the extraction procedure.

Chlorpyrifos Dissipation

Chlorpyrifos dissipation after 16 weeks was significantly increased with the addition of nutrients (Figure 39). The chlorpyrifos concentration declined to 2, 25, and 17% of the initial 4569 ± 274 μg/g for the CMA, VOA, and NA treatments, respectively (Figure 39). The DT$_{50}$ values for chlorpyrifos dissipation were calculated to be approximately 1.7 weeks ($r^2 = 0.99$) in CMA bioreactors, 8.9 weeks ($r^2 = 0.98$) in VOA bioreactors, and 6.2 weeks ($r^2 = 0.99$) in the NA bioreactors. The addition of corn meal had a pronounced and significant effect on chlorpyrifos dissipation at the 4, 8, and 16 week time points (Figure 39). Even though microbial activity was high (Figure 33) during the 16 week incubation, the addition of vegetable oil had questionable effect on chlorpyrifos dissipation. The dissipation of chlorpyrifos after 16 weeks was significantly similar in VOA and NA bioreactors.

Amending chlorpyrifos bioreactors with corn meal significantly increased the dissipation of chlorpyrifos after 4, 8, and 16 weeks compared to bioreactors not amended at all ($P = 1.6 \times 10^{-7}$, $1.1 \times 10^{-4}$, and $7.6 \times 10^{-3}$, respectively). Chlorpyrifos in CMA bioreactors decreased from an initial concentration of 4569 ± 273 μg/g to 825 ± 38.2 after 4 weeks, 376 ± 39.5 μg/g after 8 weeks, and 84.7 ± 11.0 μg/g after 16 weeks. In NA bioreactors, the chlorpyrifos concentrations decreased more slowly to 3026 ± 70.6 μg/g after 4 weeks, 1787 ± 157 μg/g after 8 weeks, and 769 ± 173 μg/g after 16 weeks.

Chlorpyrifos concentration remained higher in VOA bioreactors than in the CMA or NA bioreactors (Figure 39). After 4 weeks, there were no significant differences
Figure 37 Concentration of Didealkylatrazine and Deethylatrazine in Compost over 16 Weeks

Treatment (amendment to bioreactor)

- **0-Time**
- **Vegetable Oil**
- **Com Meal**
- **Not Amended**

20 mg/g dry matrix and 160 mg/g dry matrix

Amended bioreactors also received 16 mg soluble nitrogen/g dry matrix

Vertical bars represent standard error of the mean
Figure 38 Concentration of Deisopropyl-OH-atrazine and Deethyl-OH-atrazine in Compost over 16 Weeks

<table>
<thead>
<tr>
<th>Treatment (amendment to bioreactor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-Time</td>
</tr>
<tr>
<td>20 mg/g dry matrix</td>
</tr>
</tbody>
</table>

Amended bioreactors also received 10 mg soluble nitrogen/g dry matrix

Vertical bars represent standard error of the mean
Figure 39  Concentration of Chlorpyrifos and 3,5,6-trichloro-2-pyridinol in Compost over 16 Weeks

Treatment (amendment to bioreactor)

- 0-Time
- Vegetable Oil
- Com Meal
- Not Amended

20 mg/g dry matrix  160 mg/g dry matrix

Amended bioreactors also received 16 mg soluble nitrogen/g dry matrix

Vertical bars represent standard error of the mean.
The same letters at each time point designate significant similarity (alpha = 0.05)
(P = 0.44) between the chlorpyrifos concentrations in NA and VOA bioreactors (3150 ± 133 µg/g). However, after an additional 4 weeks, chlorpyrifos declined more significantly (P = 0.006) in NA bioreactors than in VOA bioreactors (2738 ± 166 µg/g). At the end of the experiment, the concentration of chlorpyrifos was not significantly different (P = 0.09) in bioreactors amended with vegetable oil (1147 ± 64 µg/g) compared to bioreactors not amended.

It was likely that some chlorpyrifos volatilization occurred due to its moderate volatility (2.53 mPa, Howard, 1991) and temperatures between 45 and 50 °C. The results from Project VI indicate that approximately 20% of the originally applied 14C-chlorpyrifos was recovered as volatiles. The wax paper covering may have reduced the amount of chlorpyrifos volatilization because the humidity was high, which reduces the overall volatility. It is likely that some chlorpyrifos was volatilized, but the amounts were probably less than the 20% observed in the Project VI experiment.

The formation of 3,5,6-trichloro-2-pyridinol (TCP) in the chlorpyrifos bioreactors over the 16 week period was similar to pattern of HYA formation in the atrazine bioreactors (Figure 39). It is important to note that TCP was not detected (< 5 µg/g) in the samples frozen 2 hours after chlorpyrifos was added to the matrix. The TCP concentration increased in all bioreactors throughout the 16 week incubation. TCP concentrations of approximately 476 ± 189 µg/g (4 weeks), 762 ± 266 µg/g (8 weeks), and 679 ± 122 µg/g (16 weeks) were the lowest in the vegetable oil amended bioreactors (Figure 39). At 4 and 8 weeks, the TCP concentration was highest in the CMA bioreactors at 1841 ± 138 µg/g and 2080 ± 385 µg/g, respectively, followed by the NA bioreactors, having TCP concentrations of 752 ± 200 µg/g at 4 weeks and 762 ± 266 µg/g at 8 weeks. Only at the 4 week sampling time was the concentration of chlorpyrifos significantly greater in the CMA bioreactors compared to NA bioreactors (P = 0.004). After 16 weeks, the TCP concentration of 2766 ± 122 µg/g was highest in the NA bioreactors, but not significantly higher (P = 0.052) than TCP at 1375 ± 122 µg/g in the CMA bioreactors.

Unlike atrazine, the dissipation of chlorpyrifos was significantly affected by amending the bioreactors. The hydrolysis of chlorpyrifos appeared to be the main degradative pathway (Figure 39). The hydrolysis of chlorpyrifos to 3,4,5-trichloro-2-pyridinol (TCP) is accepted as a major degradative path of chlorpyrifos dissipation. The hydrolysis of chlorpyrifos to TCP has been shown to be primarily an abiotically hydrolytic process occurring under acidic or alkaline conditions (Chapman and Harris, 1980). There
have not been any reports of sorption-mediated hydrolysis of chlorpyrifos has been observed with atrazine. There is some evidence that the hydrolysis of chlorpyrifos to TCP can be increased by the presence of microbial activity (Racke et al., 1988; Miles et al., 1983).

It is interesting that the chlorpyrifos dissipation was affected most by the corn meal amendment. It is likely that a specific microbial consortium present was able to proliferate by using corn meal, and this consortium had the ability to also degrade chlorpyrifos effectively. The possibility of having different microbial consortia present depending on the carbon energy source present and the differing capabilities of the various consortia to degrade chlorpyrifos is particularly evident between the VOA and NA bioreactors (Figure 39). The microbial activity in the NA bioreactors was considerably lower than that observed in the VOA bioreactors, but there were only minor differences in chlorpyrifos dissipation between them. Evidently, some microbes were present in the NA bioreactors which could degrade the chlorpyrifos as well as the microbial consortium present in the VOA bioreactors.

Chlorpyrifos dissipation was least enhanced by amending the bioreactors with vegetable oil, but it appeared that the 3,4,5-trichloro-2-pyridinol (TCP) concentration was lowest in the VOA bioreactors (Figure 39). The TCP concentration may be somewhat inversely proportional to the chlorpyrifos levels, but by the end of the 16 weeks, the concentration chlorpyrifos in the VOA bioreactors was not significantly different compared to the concentration in the NA bioreactors. However, the TCP concentration was significantly lower in the VOA bioreactors than either of the other two treatments. Once again, it appears that there were differences between microbial consortia in the VOA, CMA, and NA treatments, and the microbes present in the VOA bioreactors were more capable of degrading TCP.

4.5.5 Conclusions

Figure 40 represents the proposed path of atrazine and chlorpyrifos degradation in the compost system. The scheme is no different than previously proposed atrazine dissipation schemes (Kaufman and Kearney, 1970; Kruger et al., 1993) or chlorpyrifos dissipation schemes (Chapman and Harris, 1980; Racke et al., 1988). Of course atrazine
Figure 40 Degradation Pathways of Atrazine and Chlorpyrifos
and chlorpyrifos can dissipate by volatilization, sorption, and bound residue formation, which will be discussed.

Atrazine dissipation by volatilization probably did not occur to any great extent considering its low vapor pressure (0.053 mPa) and that only 0.2% of the radioactivity applied as 14C-atrazine was recovered from volatile traps in the radiolabeled experiments summarized in Project VI. However, it is likely that some chlorpyrifos did volatilize in this experiment. It is unlikely that volatilization was as high as 18% determined from the radiolabeled experiments summarized in Project VI, because the bioreactors were covered to minimize volatility and loss of water from the compost. Overall chlorpyrifos may have been further reduced because humidity was high in the bioreactor head space, which reduces the degree to which a compound volatilizes (Swarzenbach et al., 1993).

The fate of atrazine and chlorpyrifos appeared to be mainly by hydrolysis to hydroxyatrazine (HYA) and 3,5,6-trichloro-2-pyridinol (TCP), respectively, which was based on parent compound dissipation and the appearance of HYA (Figure 36) and TCP (Figure 39). Between 10 and 15% of the original atrazine concentration (4750 μg/g) remained as HYA during the 16 week experiment. Between 10 and 50% of the original chlorpyrifos concentration (4569 μg/g) was found as TCP at various times during the 16 week period. The dissipation of chlorpyrifos was significantly enhanced by the microbial population present in the CMA bioreactors. The hydrolysis may have been enhanced by the microbial consortium and the enzyme complex present in the CMA bioreactors.

The population present in the atrazine bioreactors amended with corn meal also appeared to enhance atrazine dissipation, but because hydrolysis is not the only path of degradation, it is difficult to conclude that atrazine hydrolysis was enhanced by microbial activity. It was possible that the hydrolysis of atrazine was abiotic in the bioreactors, and that the enhanced atrazine dissipation observed in the corn meal amended bioreactors came about from microbially mediated dealkylation. Further studies under more controlled conditions would have to be done to discern the role microorganisms have in the hydrolysis of atrazine in this test system.

The extent of bound residue formation, was not examined in these studies. It has been shown in previous research, including Project VI, that the formation of bound residues must be considered as a dissipation component of atrazine and its metabolites. This component appears to be less important for chlorpyrifos and its metabolites in lignocellulosic matrices. After 16 weeks in CMA bioreactors, greater than 50% of the total radioactivity originally applied as 14C-atrazine was associated with the humic
substance and unextractably-bound fractions, which is summarized in Project VI. If the
bound residues are a result of covalent bonding between atrazine, its metabolites, and the
organic matrix, it essentially obliterates the original identity of the compound because it is
now part of a larger and more complex molecule, such as the process of humic substance
formation (Berry and Boyd, 1985). There is some evidence (Bollag, 1991; Khan, 1991)
that the original molecule can return to original form as further microbial degradation
occurs, but they concluded that this process is very slow and virtually undetectable.
Therefore, bound residue formation is a true dissipation pathway, as well as a viable form
of detoxification.

The dealkylated atrazine or dealkylated hydroxyatrazine metabolites are the
metabolites that most likely form covalent bonds between matrix functional groups (i.e.
carboxylic moieties and quinones) with the exposed amino group attached to the triazine
ring (Schiavon, 1988; Winkleman and Klaine, 1991a, 1991b). If dealkylated metabolites
react to form bound residues, and these reactions occurred fairly quickly in the compost,
finding them in the free form would be difficult. It is likely that bound residue formation
involving the dealkylated metabolites did occur, since only small amounts of them were
found in the compost. In other studies, the dealkylated metabolites have been detected in
soil at similar or higher concentrations than in the compost studied here (Kruger et al.
1993; Schiavon, 1988). It is interesting that they detected similar or larger concentrations
(μg/g) of the dealkylated metabolites in soil than were detected in the compost here,
especially since the initial concentration in the soil studies was approximately 5 μg/g and
the initial concentration in the compost was approximately 4750 μg/g. In the compost
studied presented here, there were proportionally large amounts of HYA detected (500 to
800 μg/g), but the dealkylated metabolites were barely detectable at concentrations less
than 3.5 μg/g (Figures 36-38). Apparently, as the dealkylated metabolites form they
quickly react to form bound residues during the humification process occurring to a large
extent in compost (Garcia et al., 1992). It is unlikely that HYA forms bound residues to
the extent as the dealkylated metabolites of atrazine. This would explain why the HYA
concentration was between 500 and 800 μg/g in the compost and appeared to increase as
atrazine dissipated.

In conclusion, it appears that with one exception, amending bioreactors designed
to degrade waste pesticides with nutrients did not greatly enhance atrazine or chlorpyrifos
dissipation during the 16 week incubation period. The exception was that amending the
bioreactors with corn meal and NPK fertilizer significantly enhanced chlorpyrifos
dissipation. Amending an already microbially active waste pesticide system modeled here with an easily metabolized energy source may not enhance atrazine or chlorpyrifos dissipation. However, considering whether or not a toxic effect will occur by adding any pesticide at high rates (5000 µg/g) to a biobased waste disposal system like the one modeled here may not be as important as being concerned with the different microbial populations that arise as a result from different nutrient sources and natural selection. It is important to consider the entire fate of a pesticide molecule in this system, be it hydrolysis, degradation in another sense, volatilization, or bound residue formation. What happens to the metabolites in this biobased system designed to destroy waste pesticide should be considered in future studies, and it may be that the presence of metabolites in high concentrations is as important as the original parent pesticide.
4.6 PROJECT VI  Fate of $^{14}$C-Atrazine and $^{14}$C-Chlorpyrifos under Laboratory Conditions in a Solid State Fermentation Bioreactors Designed as a Pesticide Waste Disposal System

4.6.1 Abstract

Research is being conducted to develop bioreactors containing lignocellulosic materials to detoxify waste pesticides based on solid state fermentation (SSF). SSF is a viable option for persons needing an effective means to detoxify pesticide waste. In this study, a bioreactor system designed to provide information on the disposal of pesticide waste was tested under laboratory conditions at 40 °C. Radiolabeled [2,4,6-s-triazine-$^{14}$C]atrazine and [2,6-pyridinyl-$^{14}$C]chlorpyrifos were formulated with AAtrex® 4L and Dursban® 4E, respectively. These initial concentrations of atrazine and chlorpyrifos in this study were representative of the concentrations that are encountered in SSF bioreactors. The initial concentrations of chlorpyrifos in the bioreactors was $4227 \pm 835$ mg atrazine/g dry matrix (mean ± standard error of the mean). Unlike the chlorpyrifos experiment, the 8 and 16 week portions of the atrazine experiment were done at separate times and initial concentrations differed. The initial atrazine concentrations were $2682 \pm 429$ and $4053 \pm 638$ mg chlorpyrifos/kg dry matrix for the 8 and 16 week experiments, respectively. Results from the 8 and 16 week atrazine experiments were comparable, because the comparison is done on a percentage basis, and all other factors in the two experiments were similar.

The fate of each pesticide was determined by quantitating the amount of radioactivity recovered in the following fractions: volatile, carbon dioxide, ethyl acetate extractable, alkali extractable, and alkali insoluble (remaining residue). Additionally, the alkali soluble fractions were partitioned into the humic acid fraction (precipitate at pH 1 to 2) and fulvic acid fraction (soluble at pH 1 to 2). Treatments for each pesticide included bioreactors amended with nutrients and unamended bioreactors containing no nutrients. Nutrient amendment consisted of 0.4 g ground corn meal and 0.5 g nitrogen in 10 g of a 1:1:1 mixture (v/v; dry weight basis) of peat moss, steam-exploded wood, and steam-exploded peanut hulls.

The percent of radioactivity recovered was based on the amount of radioactivity recovered in zero time controls frozen and extracted with the samples. In all samples
>99% of the radioactivity recovered in the zero time controls were associated with the ethyl acetate fraction. The recovery of radioactivity or mass balance for both atrazine and chlorpyrifos was above 85% of the radioactivity originally applied (ROA) (approximately 89% for both chlorpyrifos treatments; and for atrazine, 87% in the nutrient amended treatment and 95% in the unamended treatment).

The dissipation of the pesticides in the bioreactors expressed as dissipation time values for 50% parent compound disappearance (DT<sub>50</sub>) were approximately 4 weeks for atrazine in both the nutrient amended and unamended bioreactors, and approximately 10 and 16 weeks for chlorpyrifos in the nutrient amended and unamended bioreactors, respectively. After 16 weeks of incubation at 40 °C, approximately 8% (350 mg/kg) of the atrazine originally applied was recovered during an exhaustive extraction of both the nutrient amended and unamended bioreactors. After 16 weeks of incubation at 40 °C, 35 ± 1.4% (253 mg/kg) and 47 ± 2.4% (431 mg/kg) of the original amount of chlorpyrifos applied was recovered in an exhaustive ethyl acetate extraction (9 consecutive steps) from the nutrient amended and unamended bioreactors, respectively. Nutrient amendment did not significantly increase (α = 0.05) the dissipation of atrazine or chlorpyrifos (P = 0.26 and 0.15, respectively). However, there was a trend indicating that dissipation was increased by nutrient amendment, and any significance may have become more evident with increased replication.

In the chlorpyrifos bioreactor system, dissipation was due mainly by degradation processes (abiotic or biotic) or volatilization, which was primarily due to organic volatility from the bioreactors. The distribution of radioactivity in the fractions were similar between the 8 and 16 week samples. Recoveries in the polyurethane foam (PUF) traps for trapping organic volatiles were 13 ± 0.6% (nutrient amended) and 17 ± 1.7% (unamended) of the ROA. Mineralization accounted for only 1.8 and 0.5% of the ROA in the nutrient amended and unamended bioreactors, respectively. After 8 weeks, 79.9% (nutrient amended) and 74.4% (unamended) of the ROA was recovered in the matrix fractions (ethyl acetate, alkali soluble, and alkali insoluble). After 16 weeks, 72.3% (nutrient amended) and 73.1% (unamended) of the ROA was recovered in the matrix fractions. Of that matrix associated radioactivity (MAR) in the 8 and 16 week nutrient amended and unamended samples, the majority (94 to 99%) of which was recovered in the ethyl acetate fractions. It is likely that 3,5,6-trichloro-2-pyridinol (TCP), the common hydrolytic metabolite of chlorpyrifos, or other degradates were present in the ethyl acetate fractions in amounts exceeding 25% of the initial amount of chlorpyrifos applied, since only 35%
(nutrient amended) and 47% (unamended) of the ROA was accounted for as chlorpyrifos. Less than 7% of the MAR or 3 to 4% of the ROA was recovered in the alkali soluble and insoluble fractions from each treatment. The fractionation pattern suggests that chlorpyrifos and subsequent degradates, such as TCP, did not become strongly bound to the alkali soluble and insoluble fractions.

The major path of atrazine dissipation in the bioreactors was due mainly to degradation and association of atrazine or subsequent metabolites with the alkali soluble and insoluble fractions. Atrazine volatilility (organic volatile and mineralization to $^{14}$CO$_2$) were basically non-existent; < 0.2 and 1.8% of the ROA was recovered in the organic volatile and carbon dioxide fractions, respectively. The radioactivity associated with the matrix between the 8 and 16 week samples did not change. Approximately 89% of the ROA for both treatments in the 8 week samples and 86.0 ± 2.1% (nutrient amended) and 94.9 ± 7.3 (unamended) of the ROA in the 16 week samples were associated with the matrix. Recoveries of 54.7 ± 0.9% and 56.5 ± 4.6% of the ROA were found in the ethyl acetate extractions in the 8 week nutrient amended and unamended samples, respectively. These recoveries decreased to 34.5 ± 0.8% and 30.5 ± 3.8% in the 16 week samples (both treatments). After 8 and 16 weeks, however, only 10% of the parent compound atrazine originally applied in both treatments were recovered in the ethyl acetate fractions. This suggests that there were some atrazine degradates present in the ethyl acetate fractions.

The radioactivity recovered from the alkali soluble and insoluble fractions increased from about 34% of the ROA for both treatments after 8 weeks to between about 50% (nutrient amended) and 60% (unamended) of the ROA after 16 weeks. In both treatments at 8 weeks, approximately 10% of the ROA was associated with the alkali soluble fractions (humic and fulvic acid fractions) and 24% of the ROA in the alkali insoluble fractions. This radioactivity recovered from the alkali soluble fractions significantly increased in both treatments from 8 to 16 weeks to 24.9 ± 0.8% (nutrient amended) and 41.6 ± 1.6% (unamended) of the ROA was recovered in the alkali soluble and insoluble fractions after 16 weeks.

Nutrient amendment significantly affected the distribution of radioactivity in the alkali soluble and insoluble fractions. After 16 weeks, 26.7 ± 0.5% of the ROA was associated with the alkali insoluble fraction in the nutrient amended bioreactors compared to 22.8 ± 2.0% in the unamended bioreactors. The recovery of radioactivity in the fulvic and humic acid fractions after 16 weeks were 10.5 ± 0.5% and 16.5 ± 0.2% in the nutrient amended bioreactors, respectively. Conversely, in the unamended bioreactors,
26.6 ± 0.8% and 14.9 ± 0.9% of the ROA was recovered in the fulvic and humic acid fractions. It is interesting that more radioactivity was associated with the alkali soluble and insoluble fractions in the unamended bioreactors, which had less bioactivity (demonstrated as more CO₂ evolved in nutrient amended bioreactors). It is likely that a different consortia of microorganisms were present in the different treatments, which may have influenced atrazine degradation and subsequent association with the alkali soluble and insoluble fractions.

The dissipation of atrazine and chlorpyrifos in the SSF was different with respect to the half lives, paths of dissipation, and the distribution within the various fractions. SSF for pesticide disposal and detoxification appears to be more conducive for atrazine disposal than for chlorpyrifos disposal. Because the rates of dissipation and fates are different between the two pesticides, different strategies should be followed in manipulating the SSF system to improve atrazine and chlorpyrifos dissipation. Focusing on optimizing those SSF parameters that increase bioactivity and influence bound residue formation and biodegradation should be pursued to increase atrazine detoxification. On the other hand, bound residue formation was not a dissipation path for chlorpyrifos, therefore, a good strategy to improve chlorpyrifos dissipation in a SSF disposal system should involve introducing a specific consortium of microorganisms that can degrade chlorpyrifos. In conclusion, it appears that different SSF strategies may have to be followed to improve pesticide waste detoxification, depending on different pesticides, their chemistry, and their fate in a SSF system.

4.6.2 Introduction

The proper disposal of pesticide waste is an important part of pesticide usage. Pesticide waste may include excess or outdated concentrate, excess amounts left after application, and rinsates that may include the rinsates from application equipment.

Some of the common recommendations for disposal of pesticide waste include container recycling (Fitz, 1992), landflling within the constraints of Federal, State, and local laws, and containment of rinsates for further disposal. Methods of further disposal exist and are usually performed by private businesses specializing in pesticide waste disposal. Acceptable pesticide disposal involves adsorption of the pesticides to granular activated carbon and subsequent incineration or placement into a Class I hazardous landfill.
Proposed technology for alternative pesticide waste disposal is extensive and some of the methods include evaporation pits (Junk and Richard, 1984; Winterlin et al., 1984), ozonation (Kearney et al., 1984), and ozonation followed by biological treatment (Hapeman-Somich, 1992), chemical treatment (Qian et al., 1985), and methods involving biological/microbial processes (Shelton et al., 1992). Another alternative disposal method involves solid state fermentation (SSF) and has been successfully employed under research conditions (Snell Environmental Group, 1982; California Agricultural Research Inc., 1992; Mullins et al., 1992; Shanks et al., 1992; Berry et al., 1993; Rao et al. 1995).

The research presented here was performed to determine the fate of atrazine and chlorpyrifos in a laboratory scale bioreactor system based on SSF techniques designed to dispose of pesticides as described by Mullins et al. (1992). The experiments were performed using the volatile collection apparatus described by Petruska et al. (1985) and shown in Figure 41. Atrazine and chlorpyrifos were chosen in this study because they represent two of the most widely used pesticides in the United States. In addition, atrazine presents a groundwater hazard, being one of the most commonly found pesticides in U.S. groundwater (Gustafson, 1993).

It has been demonstrated that amending systems that involve microbial activity with a secondary carbon source and nutrients can increase the biodegradation rate of recalcitrant compounds (Lu and Tsai, 1993). Therefore, treatments of nutrient amended and unamended were performed during this study to determine if nutrient amendment influence rates of atrazine and chlorpyrifos dissipation and detoxification in a SSF bioreactor system.

4.6.3 Materials and Methods

Chemicals

Analytical grade 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-s-triazine (atrazine) (>99 purity), 2-hydroxy-4-ethylamino-6-isopropylamino-1,3,5-s-triazine (OH-atrazine) (>99 purity), formulated atrazine (AAtrex® 4L), and radiolabeled [2,4,6-s-triazine-14C]atrazine (3.328 MBq/mg specific activity) were supplied by Ciba-Geigy Corporation (Atlanta, GA). Analytical grade O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate (chlorpyrifos) (>99% purity), 3,5,6-trichloro-2-pyridinol (TCP),
formulated chlorpyrifos (Dursban® 4E), and radiolabeled [2,6-pyridinyl-14C]chlorpyrifos (2.191 MBq/mg specific activity) were supplied by DowElanco (Indianapolis, IN).

Unless otherwise specified, chemicals were reagent grade and solvents used were at least pesticide grade; these were FisherBrand® (Fisher Scientific, Greensboro, NC). For most of the liquid scintillation counting (LSC), Scintiverse-BD LSC cocktail (Fisher Scientific) was used in 20 mL glass scintillation vials (Kimble) and a Beckman LS-3150 T scintillation counter. All LSC was performed using the external standard counting ratio method and a quench curve prepared with quenched 14C-Beckman standards.

Bioreactor Setup and Operation

Experiments involving atrazine and chlorpyrifos were performed using a specially designed glass bioreactor (Figure 42), which contained 10 g of a 1:1:1 mixture (v/v; dry weight) of peat moss, steam-exploded wood (SEW), and steam-exploded peanut hulls (SEPH) as the SSF matrix. The steam-explosion products were supplied by the Virginia Tech Biobased Materials Center (Blacksburg, VA). The steam-explosion process described by Overend and Chornet (1987) involved a Masonite® process gun. The SEW and SEPH originated from yellow poplar (Liriodendron tulipifera L.) and the SEPH originated from peanut hulls supplied by Birdsong Inc (Petersburg, VA). To supply a microbial inoculum to each bioreactor, approximately 10% of the peat moss portion of the mixture originated from a field scale bioreactor with a history of containing either atrazine or chlorpyrifos.

Bioreactors received either no amendments to the bioreactors or 0.4 g ground corn meal (2 mm mesh) and fertilizer (0.5 g soluble nitrogen fertilizer) once every 3 or 4 weeks. Three bioreactors per treatment were conducted as replicates. Distilled water (0.5 to 1.0 mL) was added to each bioreactor once a week to keep the matrix moist, which was done to the point of saturation. After saturation, enough water evaporated within a day to restore good airspace throughout the matrices, ensuring aerobic conditions. The thickness of the matrices in the bioreactors was between 1.5 and 2 cm, which ensured that the bioreactors remained aerobic.

A target of radiolabeled [UL-triazine-14C]atrazine and AAtrex® 4L was to be made so each of the bioreactors received 0.148 MBq/bioreactor and an amount of AAtrex® 4L to result in an initial concentration of approximately 5000 mg atrazine/kg dry matrix.
Figure 42  Laboratory-Scale Bioreactor Designed for Degradation of Pesticide Waste
A target of radiolabeled [2,6-pyrindinyl-\(^{14}\)C]chlorpyrifos and Dursban\(^{\circledast}\) 4E was to be made so each of the bioreactors received 0.148 MBq/bioreactor and an amount of Dursban\(^{\circledast}\) 4E to result in an initial concentration of approximately 5000 mg chlorpyrifos/kg dry matrix. A zero time control for each pesticide tested, containing the same application of radiolabeled and unlabeled pesticide as the treatments, was kept at -20 °C until extraction. The amount of radioactivity recovered in the zero time control was considered to be the amount of radioactivity applied to each flask, since the 0.148 MBq was only a nominal target. A blank control containing no radiolabeled pesticide and not amended with nutrients was also used in this study.

Radiolabeled experiments were performed using the bioreactor system (Figure 41) described by Petruska et al. (1985). Radiolabeled \(^{14}\)C-volatiles and \(^{14}\)CO\(_2\) were trapped in polyurethane foam and KOH traps, respectively, after ambient air passed through the glass bioreactor. The air was drawn through the bioreactor and trapping system by a vacuum pump for 1.5 minutes once an hour at an average flow rate of 200 mL minute (approximately 300 mL of bioreactor air sampled/hour). The bioreactors were contained in 4 L glass battery jars surrounded by a Tygon\(^{\circledR}\) tubing coil connected to a circulating water bath maintained at 40 °C. The battery jars that housed the bioreactors were insulated in a styrofoam box, resulting in a 40 ± 2 °C incubation temperature.

An initial volatile trap of polyurethane foam (PUF) (5.5 cm diameter by 1.5 cm thick) was placed in the top of each bioreactor. A series of two or three additional PUF's (1.2 by 3.2 cm, Jaece Inc) were placed as secondary traps into glass U-tubes connected in line between the bioreactor and the KOH trap (Figure 41). All of the PUF traps were extracted twice in cyclohexane to remove impurities prior to use. The primary PUF traps in the U-tubes were taken for analysis, leaving the next secondary PUF trap as the primary trap for the following week. A new PUF trap was placed in the U-tube last in line as the second or third trap. Recovery of the of radioactivity associated with the PUF traps was accomplished by extracting the foam plugs four consecutive times with cyclohexane, followed by a separate methanol extraction. Radioactivity was quantitated by LSC of the combined cyclohexane and separately with the methanol extract.

Radiolabeled \(^{14}\)CO\(_2\) was trapped as air from the bioreactors bubbled through 3 mL of 5 M KOH in a 50 mL buret (Figure 41). The KOH solution in the burets was removed, renewed with fresh 5 M KOH every one or two weeks. Any \(^{14}\)CO\(_2\) present in the KOH solution was transferred to ethanolamine by evolving \(^{14}\)CO\(_2\) with 25 M H\(_2\)SO\(_4\) in a 20 mL scintillation vial designed as a trapping device (Petruska et al., 1985). Evolved \(^{14}\)CO\(_2\) was
then trapped in the ethanolamine coating the inside of a 20 mL scintillation vial. Fifteen mL of scintillation cocktail (100 g 2,5-diphenyloxazole, 1.25 g 1,4-bis[5-phenyl-2-oxazolyl]-benzene, 10 mL ethanol; 1L toluene) was added directly to the vial and the radioactivity was quantitated by LSC.

Analysis of Matrix

The atrazine or chlorpyrifos bioreactor matrix was exhaustively extracted (< 0.01% of radioactivity originally applied) using approximately 40 mL of ethyl acetate in a 47 mL polypropylene centrifuge tube. The radioactivity associated with the exhaustive extraction was defined as the ethyl acetate extractable fraction. Following a 12 hour equilibration period, the tubes and their contents were then placed in a sonicating water bath at ambient temperatures (20 to 25 °C) for 0.5 hours with occasional mixing using a vortex mixer. The contents were then centrifuged (2000 x g), and the supernatant was transferred via a Pasteur pipette into a 500 mL Mason® jar with a lid. The process was repeated, negating the 12 hour sitting period, until the radioactivity was less than 0.01% originally applied. The supernatant in the jars was evaporated in a fume hood, and the volume was maintained at approximately 200 mL.

Parent pesticide compounds were analyzed by high-performance thin-layer chromatography (HPTLC) of the ethyl acetate extractions using 10 cm by 10 cm normal phase silica gel type F254 or type G HPTLC plates (MERCK) as described by Judge et al. (1993). A 0.5 mL aliquot of the extract was placed into a 1.5 mL polypropylene microcentrifuge tube and taken to near dryness with a stream of nitrogen. A measured amount between 50 and 100 μL of ethyl acetate was added, the contents were mixed, and then applied on the HPTLC plate with a capillary pipette.

The solvent system used for separating atrazine and its metabolites was a one-dimensional dual-solvent system. Analyte separation involved developing the HPTLC plate with chloroform: acetone (3:2, v/v) to approximately 65 mm above the origin. A second development was performed using benzene:propanol:butanol:glacial acetic acid (1:1:0.5:0.5, v/v) in same direction to approximately 40 mm above the origin. One lane of atrazine (0.9 Rf) and OH- atrazine (0.35 Rf) at 0.5 μg/zone were separated and visualized under 254 nm light on silica gel type F254 HPTLC plates.

The extracts and standards of chlorpyrifos (0.5 Rf) and TCP (0.3 Rf) at 0.5 μg/zone were separated using a single solvent system hexane:chloroform:methanol:glacial acetic acid (5:2:1:0.3, v/v) on silica gel type G HPTLC plates. The lane of standards were
visualized at 366 nm by dipping the edge of the plate in a 1 mM 1-pyrene carboxyaldehyde (Sigma Chemicals, Chicago, IL) in methanol. Silica gel zones were scraped at each of the standards’ Rₜ-values, in between zones, and below the origin. Each scraped zone was individually placed into scintillation fluid for LSC.

The matrix remaining after the exhaustive ethyl acetate extraction was then alkali-extracted using a modified Schnitzer and Preston (1986) method involving 0.1 M NaOH. The process was completed in a similar manner as described for the solvent extraction except that no 12 hour equilibration period was used and the humic substance extraction was completed in triplicate. The radioactivity recovered after the 0.1 M NaOH extraction was defined as the alkali insoluble fraction.

The supernatant was then acidified (pH of 2) using 25 M H₂SO₄. The precipitate (humic acid fraction) was collected by centrifugation, dried, weighed, and then combusted using a Harvey combustion instrument (Vineland, NJ). The radioactivity recovered as ¹⁴CO₂ was quantitated by LSC. The radioactivity associated with the aqueous supernatant (fulvic acid fraction) was quantitated directly by LSC of aliquots.

The alkali insoluble matrix left after the ethyl acetate extraction and humic substance extractions was dried, weighed, and part or all of the matrix was combusted using a Harvey combustion instrument to determine the amount of radioactivity associated with the matrix. Radioactivity associated with the matrix after combustion was considered to be the alkali insoluble fraction.

Statistical Analyses

In general, results are presented as a mean ± the standard error of the mean. Student’s t-test was used exclusively to determine if significant differences between mean were present between two groups of data. An α-value of 0.05 was chosen as the limit for significance, and the resulting P-value is reported when possible to indicate the degree of significance or non-significance.

Calculations of parent compound half lives or DT₅₀ values were calculated using pseudo-first order rate kinetics and the percentages of parent compounds recovered from the solvent-extractable residue using HPTLC and LSC. The following equation represents the DT₅₀ calculation:

\[ DT₅₀ = \ln \frac{2}{k} \]

where the DT₅₀ is the time it takes 50% of the original amount of pesticide to dissipate, and the "k" is the DT₅₀ coefficient unique to each pesticide in each situation. The DT₅₀
coefficient \((k)\) is the slope of line from a plot of the natural log of the pesticide concentrations or percentages on the ordinate vs. their respective times on the abscissa.

4.6.4 Results

Recovery of Radioactivity

Overall experimental recoveries ranged from 87 to 95\% of the total radioactivity originally applied to atrazine and chlorpyrifos bioreactors (Tables 23 and 24). All recoveries were based on the amount of radioactivity recovered from each sample relative to radioactivity recovered from the initial conditions of the frozen zero time control.

In the atrazine experiments, the recoveries of the nutrient amended and unamended treatments were 88.8 and 89.4\% at 8 weeks, and 86.7 and 95.2\% at 16 weeks, respectively (Table 23). The experimental recovery of radioactivity for the chlorpyrifos experiments is summarized in Table 24. The chlorpyrifos experimental radioactivity recoveries of the nutrient amended and unamended treatments were similar (93.1 and 91.2\% at 8 weeks; 87.5 and 91.0\% at 16 weeks).

It is unlikely that the unrecovered radioactivity was lost during the collection or handling of the volatile or carbon dioxide traps. The \(^{14}\)CO\(_2\) or \(^{14}\)C-organic volatiles in all experiments were essentially trapped completely, since no radioactivity was recovered in the secondary or tertiary safety traps or connecting glassware/Tygon\textsuperscript{®} tubing for these fractions. Errors involving glass adsorption and variability in the accuracy of glassware and pipettes used for volumetric estimations, dilutions, and transfers during matrix extractions may have contributed to unrecovered radioactivity and mass balances less than 100\%.

Bioactivity

Figure 43 illustrates the cumulative CO\(_2\) respired over a 16 week period for the atrazine and chlorpyrifos bioreactors. Amending the bioreactors with corn meal and soluble nitrogen fertilizer significantly increased overall CO\(_2\) respiration observed in the atrazine and chlorpyrifos bioreactors, indicating increased bioactivity (atrazine \(P = 0.002\); chlorpyrifos \(P = 0.0003\)). The atrazine and chlorpyrifos unamended bioreactors evolved 672 ± 105 and 674 ± 203 mg of CO\(_2\), respectively, which were significantly similar
### Table 23
Atrazine: Percent Experimental Recovery of Radioactivity

<table>
<thead>
<tr>
<th>Fractions</th>
<th>8 Week</th>
<th>Atrazine</th>
<th>16 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-Time Control Amended Unamended</td>
<td></td>
<td>0-Time Control Amended Unamended</td>
</tr>
<tr>
<td></td>
<td>0.10 +/- 0.00 0.07 +/- 0.00</td>
<td>na 0.21 +/- 0.15 0.10 +/- 0.03</td>
<td>na 0.23 +/- 0.17 0.06 +/- 0.04</td>
</tr>
<tr>
<td>Organic Volatiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>Ethyl Acetate Extract na 98.3 54.66 56.46 +/- 0.88 +/- 4.55</td>
<td>na 94.93 34.48 30.51 +/- 0.75 +/- 3.75</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkali Soluble</td>
<td>1.17 9.85 9.25 na +/- 0.85 +/- 2.26</td>
<td>na 4.33 24.88 41.55 +/- 0.76 +/- 1.60</td>
<td></td>
</tr>
<tr>
<td>Alkali Insoluble</td>
<td>0.53 23.98 23.53 na +/- 0.85 +/- 1.35</td>
<td>na 0.73 26.67 22.79 +/- 0.54 +/- 1.97</td>
<td></td>
</tr>
<tr>
<td>Matrix Total</td>
<td>100 88.49 89.24 +/- 2.58 +/- 8.16</td>
<td>99.99 86.03 94.85 +/- 2.05 +/- 7.32</td>
<td></td>
</tr>
<tr>
<td>Total Recovery</td>
<td>100 88.80 89.41</td>
<td>100 86.68 95.18</td>
<td></td>
</tr>
<tr>
<td>Radioactivity</td>
<td>0.145 0.129 0.131</td>
<td>0.086 0.075 0.082</td>
<td></td>
</tr>
<tr>
<td>Recovered</td>
<td>MBq MBq MBq</td>
<td>MBq MBq MBq</td>
<td></td>
</tr>
</tbody>
</table>
### Table 24: Chlorpyrifos: Percent Experimental Recovery of Radioactivity

<table>
<thead>
<tr>
<th>Fractions</th>
<th>8 Week</th>
<th>16 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-Time Control</td>
<td>Amended</td>
</tr>
<tr>
<td></td>
<td>+/− sem²</td>
<td>+/− sem</td>
</tr>
<tr>
<td>Organic Volatiles</td>
<td>na</td>
<td>12.76 +/− 0.70</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>na</td>
<td>0.52 +/− 0.07</td>
</tr>
<tr>
<td>Matrix</td>
<td>Ethyl Acetate Extract¹</td>
<td>99.93 79.26 74.10</td>
</tr>
<tr>
<td></td>
<td>na +/− 3.39</td>
<td>+/− 1.75</td>
</tr>
<tr>
<td></td>
<td>Alkali Soluble¹</td>
<td>0.00003 0.0006 0.0003</td>
</tr>
<tr>
<td></td>
<td>na</td>
<td>na 0.07 0.60 0.30</td>
</tr>
<tr>
<td></td>
<td>Alkali Insoluble¹</td>
<td>0.07 +/− 0.08</td>
</tr>
<tr>
<td></td>
<td>Matrix Total</td>
<td>100 79.86 74.40</td>
</tr>
<tr>
<td></td>
<td>+/− 4.24 +/− 2.36</td>
<td>+/− 1.84</td>
</tr>
<tr>
<td>Total Recovery</td>
<td>100 93.14 91.20</td>
<td>+/− 4.24 +/− 2.36</td>
</tr>
<tr>
<td></td>
<td>+/− 4.24 +/− 2.36</td>
<td>+/− 2.36</td>
</tr>
<tr>
<td>Radioactivity</td>
<td>0.119 mBq mBq mBq</td>
<td>0.111 mBq mBq mBq</td>
</tr>
<tr>
<td>Recovered</td>
<td>mBq mBq mBq</td>
<td>mBq mBq mBq</td>
</tr>
</tbody>
</table>

¹Percentages of radioactivity based on radioactivity recovered from 0-Time Control as 100%

²Nominal target to apply was 0.145 MBq. Actual chlorpyrifos conc. was 2682 µg/g for the 8 week exp. and 4053 µg/g for the 16 week exp. The 8 & 16 week experiment were done at different times.

The different initial concentrations should not affect results due to percentage basis for comparison.

²sem = Standard error of the mean

³radioactivity recovered in the ethyl acetate extraction

⁴radioactivity recovered in the alkali soluble fractions, which include humic and fulvic acid fractions

⁵radioactivity recovered after combustion of remaining matrix or alkali insoluble fraction
Figure 43  Cumulative Carbon Dioxide Evolution In Atrazine and Chlorpyrifos Bioreactors

Each datum represents a cumulative mean of CO₂ evolution to that point for 3 to 4 bioreactors.

Vertical Bars represent standard error of the mean.
(P = 0.7) (Figure 43). In the nutrient amended bioreactors after 16 weeks accumulation, there were no significant differences (P = 0.7) between 1768 ± 164 mg CO₂ evolved from the atrazine bioreactors and 1903 ± 215 mg CO₂ evolved from the chlorpyrifos bioreactors.

Figure 43 shows the timepoints when corn meal and nitrogen fertilizer were added to the bioreactors. Once every 4 weeks, there was a sharp increase in CO₂ evolution. The majority of increased CO₂ evolution appeared to occur during the first week after amending the bioreactors.

14CO₂ Recovery

The cumulative amount of 14CO₂ recovered from the bioreactors for both pesticides over 8 and 16 weeks did not exceed 2% of the radioactivity originally applied (Tables 23 and 24). Following 8 weeks of incubation at 40 °C, the cumulative 14CO₂ recovered from atrazine and chlorpyrifos containing bioreactors was significant when amended with nutrients (atrazine, P = 0.0003; chlorpyrifos, P = 0.013). The 14CO₂ accumulated over 16 weeks for chlorpyrifos was also significantly greater in nutrient amended atrazine bioreactors (P = 0.005). However, after 16 weeks incubation, no significant differences (P = 0.22) were observed for 14CO₂ recovered between nutrient amended and unamended bioreactors. One of the unamended atrazine bioreactors after 16 weeks incubation had produced approximately three times the 14CO₂ as the other replicates. If this datum is removed from the statistical analysis, there is a significant difference between nutrient amended and unamended bioreactors (P = 0.02).

14C-Organic Volatile Recovery

As shown in Tables 23 and 24, the radioactivity recovered in the organic volatile fraction from the polyurethane foam traps (PUF) was considerably higher in the chlorpyrifos bioreactors than in the atrazine bioreactors. From the atrazine bioreactors over 16 weeks, between 0.06% and 0.23% of the radioactivity originally applied was recovered in all PUF traps, whereas from the chlorpyrifos bioreactors between 13.4% and 17.5% of the radioactivity originally applied was recovered in the PUF traps.

Between 95% and 100% of the radioactivity recovered in the polyurethane foam traps in the atrazine and chlorpyrifos experiments was recovered in the primary PUF
placed in the top of the bioreactors. No radioactivity (< twice background dpm) was recovered in any case from the secondary U-tube PUF traps.

The efficiency of the cyclohexane/methanol extractions were approximately 100%. Radioactivity bound to the PUF traps recovered after extraction was only two to three times background radioactivity (20-40 dpm). The bound radioactivity was determined by dissolving the primary bioreactor PUF's in a closed glass jar with 25 M H$_2$SO$_4$ for a minimum of 48 hours, neutralizing the pH with 5 M KOH, and quantitating the radioactivity of an aliquot by LSC.

The majority of the radioactivity recovered in the organic volatile fractions of both the atrazine and chlorpyrifos experiments was recovered between the experiment initiation and the 8 week point. For both pesticides, there appeared to be only a slight increase in the radioactivity recovered in the organic volatile fraction between 8 and 16 weeks. In all cases this slight increase observed between the 8 and 16 week recoveries was not significant (atrazine: nutrient amended, $P = 0.82$; unamended, $P = 0.19$; chlorpyrifos: nutrient amended, $P = 0.10$; unamended, $P = 0.87$).

The radioactivity recovered from the organic volatile fractions of the nutrient-amended bioreactors appeared to be higher than the unamended bioreactors in both the atrazine and chlorpyrifos experiments. However, the only significant difference observed between the organic volatile fractions from nutrient amended and unamended bioreactors was in the 8 week chlorpyrifos experiment ($P = 0.0035$).

**Recovery of Radioactivity from the Matrix**

The majority of radioactivity recovered from the atrazine and chlorpyrifos bioreactors was associated with the matrix material, which included ethyl acetate extractable, alkali soluble, and alkali insoluble fractions (Table 23 for atrazine and Table 24 for chlorpyrifos). In the atrazine experiments over 8 and 16 weeks, between 86% and 95% of the radioactivity originally applied (ROA) was associated with matrix. However, due to volatilization observed (13.4 to 17.5% of the ROA) in the chlorpyrifos bioreactors, between 72% and 80% of the ROA was recovered in the matrix fraction. The majority of the ROA in the atrazine and chlorpyrifos frozen zero time controls was recovered in the ethyl acetate extraction fraction, which exceeded 95% and 99% of the ROA for atrazine and chlorpyrifos, respectively (Tables 23 and 24).
The presentation of the radioactivity recovered from each matrix fraction as values of the ROA is appropriate for discussing experimental recovery and overall dissipation. However, it is difficult to compare the distribution of radioactivity in the various matrix fractions (ethyl acetate, alkali soluble, and alkali insoluble fractions) with different experimental recoveries and varying amounts of volatility. Where appropriate, the distribution of the radioactivity in the matrix fractions will be presented as a percentage of the total radioactivity recovered from the matrix for comparative purposes. In other words, the distribution of radioactivity in the matrix will be expressed as a percentage of the matrix associated radioactivity or MAR.

**Ethyl Acetate Extractable Pesticide Residue**

The lignocellulosic materials in bioreactors were exhaustively extracted using ethyl acetate until radioactivity was below 150 dpm/mL in the supernatant (40 mL). Following the ethyl acetate extraction procedure, no significant radioactivity (< 0.01 of the ROA) was recovered with an additional methanol/ H₂O (80:20, v/v) wash. Extraction efforts were discontinued following 14 consecutive ethyl acetate extractions for atrazine and 9 extractions for chlorpyrifos.

Radioactive amounts of 54.7 ± 0.9% and 56.5 ± 4.6% were recovered in the ethyl acetate extract from the nutrient amended and unamended atrazine bioreactors after 8 weeks. In the 16 week atrazine nutrient amended and unamended bioreactors, between 34.5 ± 0.7% and 30.5 ± 3.7% of the ROA were recovered in the ethyl acetate extraction (Table 24 and Figure 44). It is interesting that the majority of the radioactivity associated with the matrix (>94% of the MAR) in the chlorpyrifos bioreactors after 8 and 16 weeks was recovered in the ethyl acetate extraction (Table 24 and Figure 45).

Ethyl acetate extractable atrazine from both treatments decreased from the initial 100% applied to between 10 to 12% of the ROA after 8 weeks. After an additional 8 week’s incubation, the atrazine recovered from the ethyl acetate extraction appeared to decrease only slightly to approximately 8 to 9% of the ROA. Nutrient addition did not affect the rate of atrazine dissipation between 8 and 16 weeks (8 weeks, P = 0.63; 16 weeks, P = 0.26) (Figure 46).

Ethyl acetate extractable chlorpyrifos decreased over 8 and 16 weeks (Figure 46), but not to the extent that was observed in the atrazine bioreactors. After 8 weeks, the amounts decreased to approximately 80% of the ROA with no significant differences
Figure 44. Atrazine Matrix Fractions: Distribution of Radioactivity

Values are % of radioactivity with respect to what was recovered in the zero time control ± standard error of the mean
(Nominal target to apply was 0.145 MBq and 5000 µg/g atrazine)
8 Week Radiolabeled Matrix Fractions

Alkali-Insoluble 0.6 ± 0.1
FA + HA = 0.0006

79.3 ± 3.4
Nutrient-Amended

Alkali-Insoluble 0.3 ± 0.1
FA + HA = 0.003

74.1 ± 1.8
Unamended

Alkali-Insoluble 0.1

99.9
0-Time Control

16 Week Radiolabeled Matrix Fractions

Alkali-Insoluble 2.1 ± 1.0
FA 0.9 ± 0.3
HA 1.5 ± 0.4

67.8 ± 0.7
Nutrient-Amended

Alkali-Insoluble 1.5 ± 1.2
FA 0.1 ± 0.02
HA 2.1 ± 1.8

69.3 ± 0.7
Unamended

Alkali-Insoluble 0.1

99.9
0-Time Control

Legend:
- Alkali-Insoluble
- Fulvic Acid
- Humic Acid
- Ethyl Acetate Extracted

Figure 45. Chlorpyrifos Matrix Fractions: Distribution of Radioactivity

Values are % of radioactivity with respect to what was recovered in the zero time control ± standard error of the mean
(Nominal target to apply was 0.145 MBq and 5000 µg/g atrazine)
(P = 0.11) between the nutrient amended and unamended bioreactors (76 ± 3.0% and 84 ± 3.4%, respectively). After an additional 8 week's incubation, the radioactivity recovered in the ethyl acetate extraction as chlorpyrifos was 35 ± 1.4% of the ROA in the nutrient amended bioreactors and 47 ± 2.4% in the unamended bioreactors with no significant differences between the treatments (P = 0.15). The observed decrease of ethyl acetate extractable chlorpyrifos between 8 and 16 weeks (Figure 46) was significant between the nutrient amended and unamended bioreactors (P = 0.004, nutrient amended; P = 0.006, unamended).

The parent pesticide compounds were quantitated by LSC of the zonal scrapings from an HPTLC plate after separating the ethyl acetate extractable pesticide residues by HPTLC methods. The results are illustrated in Figure 46. Based on the specific activity and the recovered radioactivity at the respective Rp, the initial concentrations of chlorpyrifos was 4227 ± 835 mg pesticide/kg dry matrix. The 8 and 16 week samples in the chlorpyrifos experiment were applied to with the same radioactive/ Dursban® 4E mixture. However, the 8 and 16 week samples in the atrazine experiment were applied to at separate times. Unfortunately, the amount of radioactivity and AAtrix® 4L was different in the 8 and 16 week samples. The atrazine experiments were done at separate times, and the initial concentrations were 2682 ± 429 mg/kg at 8 weeks and 4053 ± 638 mg/kg at 16 weeks. These differences should not have affected the ability to compare the two sampling time experiments. Radioactivity recovered in the various fractions is compared on a relative basis. Any affects on bioactivity should be similar between samples with different initial concentrations. Both are well above field applications and effects on bioactivity would be similar due such high concentrations.

**Alkali Soluble Radioactive Fractions**

The radioactivity recovered with the alkali soluble fractions (humic and fulvic acid fractions) was considerably greater (P < 0.001) in the atrazine bioreactors than in the chlorpyrifos bioreactors after 8 and 16 weeks (Figures 44 and 45). Less than 0.1% of the ROA in the nutrient amended and unamended chlorpyrifos bioreactors was recovered after 8 weeks in the alkali soluble fractions (Table 24). This increased to approximately 2% after 16 weeks for both chlorpyrifos treatments (nutrient amended and unamended bioreactors). In the chlorpyrifos zero time frozen controls at 8 and 16 weeks, basically no radioactivity was recovered with the alkali soluble fractions.
Figure 46. Ethyl Acetate Extractable Parent Pesticide Dissipation from Bioreactor Matrices

Vertical lines on bars represent standard error of the mean.
Bar Values are % of original 0-time values.
After 8 weeks, between 9% and 10% of the ROA in both atrazine treatments was recovered in the alkali soluble fractions (Table 23). For both treatments, the radioactivity was about evenly split between the humic and fulvic acid fractions (Figure 44). Between 8 and 16 weeks, an increase of the radioactivity recovered in the alkali soluble fractions was observed (Table 23). After 16 weeks, the ROA recovered in the alkali soluble fractions in the atrazine unamended bioreactors (41.6 ± 1.6%) was significantly greater (P = 0.017) than 24.9 ± 0.8% recovered from the nutrient amended bioreactors.

After 16 weeks in the atrazine bioreactors, it should be noted that radioactivity was distributed differently in the humic and fulvic acid fractions between the nutrient amended and unamended treatments (Figure 44). In the 16 week nutrient amended bioreactors, 16.5 ± 0.2% of the ROA recovered in the humic acid fraction was significantly greater (P = 0.048) than 10.5 ± 0.5% ROA recovered in the fulvic acid fraction. The opposite was true in the unamended bioreactors, where 26.6 ± 0.8% of the ROA in the fulvic acid fraction was significantly greater (P = 0.002) than 14.9 ± 1.6% of the ROA in the humic acid fraction.

**Alkali Insoluble Radioactive Residue**

After 8 weeks, the radioactivity recovered from the alkali insoluble fractions by combustion in the chlorpyrifos bioreactors was less than 0.1% of the ROA. The percent radioactivity recovered in the matrix (MAR) increased only slightly after 16 weeks to 2.8 ± 1.4% and 2.0 ± 1.4% of the ROA in the nutrient amended and unamended bioreactors (Figure 45).

A larger proportion of the MAR was recovered in the alkali insoluble fractions in the atrazine bioreactors compared to the chlorpyrifos bioreactors (Figures 44 and 45). After 8 weeks, the radioactivity recovered in the alkali insoluble fractions of the atrazine nutrient amended and unamended bioreactors was 24 ± 0.8% and 23.9 ± 1.0%. After an additional 8 weeks’ incubation, there was only a slight increase of radioactivity recovered in the alkali insoluble fraction (26.7 ± 0.5% of the ROA in the nutrient amended versus 22.8 ± 2.0% MAR, unamended). The difference of radioactivity associated with the alkali insoluble fraction was significantly greater in the nutrient amended bioreactors (P = 0.027).
4.6.5 Discussion

Atrazine dissipated from the bioreactors at a faster rate than did chlorpyrifos in the laboratory scale bioreactor system designed to evaluate the fate of pesticides in a lignocellulosic matrix during solid state fermentation. The dissipation time for 50% parent compound disappearance (DT$_{50}$) represents the decline of parent pesticide compound recovery in the ethyl acetate extraction. DT$_{50}$ values were determined assuming pseudo first-order kinetics, and coefficients of determination ($r^2$) ranged from 0.90 to 0.96.

The DT$_{50}$ of atrazine was approximately 4 weeks, and amending the atrazine bioreactors with nutrients did not significantly affect the DT$_{50}$ (P values > 0.25). DT$_{50}$ values have been reported to be between less than a week to approximately 24 weeks for atrazine in soil applied at approximately 5 mg/kg (field application rate) (Goring et al., 1975). Winkleman and Klaine (1991a) reported that atrazine decreased to less than 1% of the initial 5.3 mg/kg rate after 180 days in an acidic (pH 5.3) Tennessee silt loam.

Chlorpyrifos had DT$_{50}$ values of approximately 10.5 and 16.5 weeks in the nutrient amended and unamended treatments, and there were no significant DT$_{50}$ differences between chlorpyrifos treatments (P = 0.11). Other researchers have observed that DT$_{50}$ values for chlorpyrifos in soil varied with soil type, pH, moisture content, initial concentration, formulation type, and biological activity. DT$_{50}$ values were reported to be less than one week in sandy loam under laboratory conditions using technical chlorpyrifos at normal field rates of less than 10 mg/kg (Miles et al., 1979) to approximately 4.3 years in soil that had termite idle application of 1000 mg/kg (Racke et al., 1994).

The concentrations of atrazine and chlorpyrifos in the bioreactors in this study were several times greater than what is used in normal soil application rates, which reflect the concentrations that would be encountered in the pesticide waste disposal system. However, Berry et al. (1993) and Rao et al. (1995) have performed laboratory experiments involving atrazine in a similar SSF bioreactor system with lignocellulosic materials. Berry et al. (1993) reported that after 480 days incubation, atrazine degraded to 0.2% and 0.05% of approximately 7000 mg/kg atrazine initially applied to peat moss and steam-exploded wood SSF systems, respectively. Rao et al. (1995) reported that all of the atrazine applied to the bioreactors had dissipated after 160 days at 37 °C.

In the chlorpyrifos bioreactors, dissipation was mainly due to degradation (abiotic and/or biotic) and by volatilization. Chlorpyrifos, having a vapor pressure of 2.53 mPa (Howard, 1991), is a more volatile pesticide than atrazine which has a vapor pressure of
0.037 mPa (Howard, 1991). With a moderately volatile pesticide like chlorpyrifos, maintaining the bioreactor temperature at 40 °C and mixing the bioreactors weekly would explain why between 12.8 and 17.5% of the radioactivity originally applied was recovered in the organic volatile fraction after 16 weeks (Table 24). Considerable volatilization of pesticides do occur. Trifluralin is considered to be a volatile pesticide (15 mPa, 20 °C) (Taylor and Spencer, 1990) being about 6 times more volatile than chlorpyrifos. White et al. (1977) observed a 26% loss of originally applied trifluralin by volatilization after 9 days and 90% loss by volatilization after 35 days.

Even though the identity of the pesticide or metabolites comprising the radioactivity recovered in the PUF traps (organic volatile traps) was not determined, it is likely that the majority of the radioactivity recovered in the PUF traps was chlorpyrifos, not the primary metabolite 3,5,6-trichloro-2-pyridinol (TCP). TCP has a vapor pressure of 0.013 mPa (Meikle and Hamaker, 1981), which makes it less volatile than chlorpyrifos (2.53 mPa).

As shown in Table 24, the majority of chlorpyrifos volatilization occurred between experiment initiation and 8 weeks with only a small increase in volatilization between 8 and 16 weeks. Volatilization of chlorpyrifos may have decreased early in the experiment as more chlorpyrifos became sorbed to the matrix. It is known that the volatilization of a pesticide can be slowed due to sorption processes (Spencer, 1987). Volatility has been demonstrated to have an inverse relationship with sorbent surface area (Guenzi and Beard, 1970). Additionally, the combination of increased organic matter content and increased moisture content can decrease actual volatility (Dörfler et al., 1991).

Volatilization of chlorpyrifos tended to be greater in the unamended bioreactors compared to the nutrient amended ones; conversely, there was an indication that overall chlorpyrifos dissipation was greater in the nutrient amended bioreactors. Chlorpyrifos degradation appeared to be enhanced by increased bioactivity (i.e. increased ¹⁴CO₂ release) as was observed in nutrient amended bioreactors (Figure 43). This may explain why overall volatility was slightly greater in the unamended bioreactors. If a larger proportion of chlorpyrifos was being degraded by microbiially mediated metabolism, then there would have been a proportionally less amount of chlorpyrifos available for volatilization.

The majority of chlorpyrifos dissipation from the bioreactors, not associated with volatilization, was due to degradation (biotically mediated or abiotic). After 16 weeks of incubation at 40 °C, the recovery of the parent compound chlorpyrifos from the matrix in
the ethyl acetate extraction was reduced to 35% and 47% of the original amount applied in the nutrient amended and unamended bioreactors, respectively (Figure 46). The degradation of chlorpyrifos that occurred in the bioreactors was most likely by hydrolysis to 3,5,6-trichloro-2-pyridinol [TCP], which has been demonstrated as the major degradative pathway (Chapman and Harris, 1980; Howard, 1991; Cink and Coats, 1993; and Chapter V). TCP was found in bioreactors in Project V of this Dissertation at levels approximately 25% of the initial atrazine concentration of approximately 4750 μg/g. Chlorpyrifos hydrolysis has been shown to be a primarily abiotic process, although microorganisms can influence the hydrolysis to TCP (Miles et al. 1983; Racke et al. 1990). In sterile buffered solutions and in autoclaved soil, chlorpyrifos appeared to be most stable in acidic to neutral pH environments (Howard, 1991), but hydrolysis rates increase as the pH becomes more alkaline (Meikle and Youngson, 1978; Macalady and Wolfe, 1983).

The pH of the lignocellulosic matrix in the bioreactors ranged between 5 and 6.5 in this study, suggesting that microbial activity may have had a role in the hydrolysis of chlorpyrifos.

Mineralization in the chlorpyrifos bioreactors after 16 weeks was low (0.5 to 1.8% of the radioactivity originally applied); there was significantly more mineralization to $^{14}$CO$_2$ in the nutrient amended bioreactors (Table 24). Nutrient amendment increased microbial activity as indicated by the increased overall CO$_2$ evolution (Figure 43). The influence of adding nutrients on the bioreactors, although insignificant ($P = 0.15$), tended to increase chlorpyrifos degradation (Figure 46); it was concluded in Project V that similar nutrient amendment significantly increased chlorpyrifos degradation. It seems likely that a larger population of microorganisms present in those bioreactors amended with nutrients would be capable of hydrolyzing a larger amount of chlorpyrifos at the pyridinyl moiety.

Between 93.9 and 99.6% of the matrix associated radioactivity (MAR) in the chlorpyrifos bioreactors was recovered in the ethyl acetate extraction after 8 and 16 weeks (Figure 45), suggesting that the association of the parent compound or degradates with the lignocellulosic matrix was primarily by physical sorption. It has been demonstrated that phenolic compounds can become bound to soil organic matter during humification (Taylor and Battersby, 1967, Stevenson, 1982) through covalent bonding at the hydroxy carbon of the phenolic species (Berry and Boyd, 1984). However, in this study after 16 weeks, the pyridinyl moiety of TCP formed from the hydrolysis of chlorpyrifos did not appear to become covalently bound to the organic. In another study (Racke et al., 1994), chlorpyrifos hydrolysis occurred in soil with an initial concentration of approximately 1000
mg/kg chlorpyrifos, however, there was only minimal unextractably bound radioactivity as observed in this study. Two explanations offered by Racke et al. (1994) for the lack of TCP degradation and unextractably bound radioactivity were that the sites for TCP degradation are limited in soil (whether abiotic or biotic), and that the presence of TCP at elevated concentrations is toxic to those soil microorganisms that can normally degrade TCP and mediate covalent binding to the soil organic matter.

There are three major degradative reactions that occurred during the degradation of chlorpyrifos of which the presence of TCP can affect in the bioreactor. These reactions include the hydrolysis of chlorpyrifos to TCP, the further metabolism of TCP, and the unextractable association of TCP to the soil organic matter during humification. Even though chlorpyrifos degradation can be influenced by microbially mediated reactions (Miles et al., 1983), it has been demonstrated that enhanced degradation may not actually occur when the chlorpyrifos concentration is much above the normal agricultural field application rate of 5 to 10 mg/kg, which is directly due to the amount of TCP formed (Racke et al., 1988; Somasundaram et al., 1990; Cink and Coats, 1993). TCP concentrations above 100 mg/kg were shown to completely inhibit the normal microbially-mediated degradation of isophenphos and carbofuran (Racke et al., 1988). It was concluded in Project V that elevated TCP concentrations did not adversely affect microbial activity under SSF conditions in the bioreactors. Additionally, in this study and in the Project V study, it appeared that increased microbial activity after nutrient amendment (Figure 43) increased chlorpyrifos degradation (Figure 46). It is difficult to conclude whether or not any microbially mediated TCP degradation was adversely affected by elevated TCP concentrations. The contradiction between the adverse effect caused by TCP in soil-based environments versus the lignocellulosic environment may be best explained by the differences of microbial activity between the two environments. The microbial activity in the bioreactors under SSF conditions was greater when compared to soil. The microbial activity, indicated as CO2 evolution, was 8 and 140 μg CO2/g·h in the unamended and nutrient amended bioreactors, respectively, compared to 0.2 and 20 μg CO2/g·h in unamended and nutrient amended soil microcosms without pesticide (Ritz et al., 1992). The lignocellulosic matrix and nutrient amendment supplies more usable energy for microbial populations in the bioreactors, whereas soil environments have limited energy resources. Any toxic effect which might have been present in the bioreactors and affecting certain types of microorganisms appeared to be occluded by
microorganisms that were not adversely affected, which was indicated as increased microbial activity.

Increased microbial activity and any resulting increased enzyme amounts present in the bioreactors did not appear to influence the extent that chlorpyrifos or degradates became unextractably associated with the organic matter during humification. This would lead to the conclusion that the extent of TCP binding to organic matter is limited, which may be due to a limited number of catalysis sites occur as suggested by Racke et al. (1990). It would seem likely that increased microbial activity would confer more catalysis, but this does not appear to be case in this study.

A plausible explanation for the lack of radioactivity recovered in the alkali soluble and insoluble fractions is that TCP is not compatible with the extracellular enzymes which are involved with humification. Substituted phenolics are common soil compounds involved with humification, and they would be primarily in the enol form at neutral pH (Taylor and Battersby, 1967; Martin and Haider, 1971). Monosubstituted phenols have been demonstrated to polymerize with other phenolics in enzyme mediated reactions during humification (Berry and Boyd, 1984; 1985). They also concluded that electron-withdrawing constituents present on the phenol, such as halogens reduce the reactivity of the phenolic compound (Berry and Boyd, 1984). Pyridinols are similar to phenols, but in actuality they must have different enough qualities to prevent them from being compatible with humification enzymes. Tautomerism occurs with TCP and the majority of TCP would be in the keto form rather than the enol form. Additionnally, the pyridinol ring is substituted with three electron-withdrawing chlorine atoms. The combination of the keto form and the presence of chlorines must hinder reactivity.

In the atrazine bioreactors, the major path of atrazine dissipation in the bioreactors was due mainly to degradation, and atrazine or its metabolites becoming unextractably associated with the alkali soluble and alakali insoluble fractions. Atrazine volatilization did not occur to any appreciable amount; < 0.2% of the ROA was recovered in the organic volatile fraction over the 16 week incubation period. The fate of atrazine by mineralization was also minimal over 16 weeks, ranging from 0.23 to 1.75% of the ROA.

Atrazine degradation can be mediated by abiotic or biotic reactions. It has been demonstrated that hydrolysis of atrazine can occur abiotically by association with clay surfaces (Armstrong et al., 1967) and interactions with unionized carboxyl groups present in soil organic matter (Gamble and Khan, 1990; Armstrong and Chesters, 1968). The dealkylation of atrazine is another major degradation route of atrazine, which has been
shown to be mainly a microbially mediated process, and to a lesser extent, an abiotic process (Winkleman and Klaine, 1991a). It is reported that further degradation and subsequent mineralization of the hydrolytic and dealkylated products are primarily biotic (Kruger et al., 1993).

The atrazine DT$_{50}$ in this study was approximately 4 weeks, and after 8 weeks only 10% of the original amount of atrazine applied could be recovered as the parent compound in the ethyl acetate extract (Figure 46). The analysis of the ethyl acetate extract involved only the quantitation of parent compound, atrazine, not metabolite quantitation. The amount of the parent compound atrazine recovered in the ethyl acetate extract (10%) did not change between 8 and 16 weeks (Figure 46). However, the percentage of the radioactivity in the ethyl acetate extraction fraction decreased from approximately 55 and 56% of the ROA in the nutrient amended and unamended bioreactors, respectively, to between 35 and 31% of the ROA, respectively (Table 23). Atrazine metabolites that had formed and were present at 8 weeks must have become associated with the alkali soluble and alkali insoluble fractions in a way that prevented their recovery during the ethyl acetate extraction after 16 weeks.

Even though microbial activity did not affect the rate of atrazine degradation (Figure 46), the differences in microbial activity in the nutrient amended and unamended bioreactors did appear to influence the distribution of the radioactivity in the alkali soluble and insoluble fractions. More radioactivity was associated in the alkali soluble and insoluble fractions in the 16 week unamended bioreactors (63.3% of the ROA, Table 23) than in the nutrient amended bioreactors (50.5% of the ROA, Table 23). Cook et al. (1995) performed a similar study involving the fate of $^{14}$C-atrazine in SSF bioreactors. They compared bioreactors at composting temperatures (35 to 40 °C) and room temperature (25 °C). The results indicated that differences in microbial activity in the composted and room temperature composts influenced atrazine degradation and the distribution of radioactivity in the matrix. Approximately 40% of the ROA was recovered associated with the matrix in the higher temperature composted bioreactors compared to 15% of the ROA in the room temperature bioreactors. They did not perform an alkali soluble extraction, therefore, the recoveries were equivalent to the combination of the alkali soluble and insoluble fractions reported for this study. Cook et al. (1995) also reported that atrazine dissipation was greater in the cooler room temperature bioreactors with a recovery of 20 - 25% of the original amount of atrazine applied. This compared to approximately 40% of the original amount of atrazine applied that was recovered in the
composted bioreactors. It is interesting that Cook et al. (1995) observed greater atrazine dissipation in the cooler bioreactors than in composting situation with higher temperatures. Their results are not directly comparable to the results in this study, but in both studies, differences in microbial activity and in the microbial populations in each situation did appear to influence the fate of atrazine.

In this study, there was a distinct difference between the atrazine and chlorpyrifos experiments in the association of radioactivity with the alkali soluble fractions (humic/fulvic acid fractions) and alkali insoluble fractions (Figures 44 and 45). As shown in Table 16 and Figure 44, an increasing amount of radioactivity recovered in the atrazine bioreactors became tightly associated with the alkali soluble and insoluble fractions. However, only small amounts of radioactivity in the chlorpyrifos bioreactors over 16 weeks became associated with the alkali soluble and alkali insoluble fractions (Table 24 and Figure 45).

After 16 weeks in the atrazine bioreactors, there was a bit more radioactivity associated with the alkali insoluble fraction in the nutrient amended bioreactors (26.7% of the ROA) compared to the unamended bioreactors (22.8% of the ROA) (Figure 44). Conversely, there was there was a significantly larger amount of radioactivity associated with the fulvic acid fraction of the unamended versus the nutrient amended bioreactors. Singh and Amberger (1990) found that the amount of fulvic acid recovered increased in lignocellulosic compost amended with a carbohydrate source. It was not evident in their test system whether the effect was chemically based or related to increased microbial activity due to a readily usable energy source, such as glucose.

The increase in atrazine radioactivity associated with the fulvic acid fraction from the 16 week unamended matrix may have been a direct result of increased microbial metabolism, since the overall microbial activity was greater in the nutrient amended bioreactors than in the unamended bioreactors (Figure 43). Another influence may have involved the corn meal and NPK fertilizer itself, changing the type of organic material involved in humification.

In order for the microorganisms present in either type of bioreactor to have used lignocellulosic materials catabolically, they must have possessed a specific enzyme complex capable of degrading lignocellulosic materials. The microbial consortium present in the unamended bioreactors (i.e. no cornmeal addition), where lignocellulosic materials were the only source for microbial catabolism, may have been different than the microbial consortium present in the nutrient amended bioreactors. It is not clear what the
mechanisms were of atrazine association with the humic substance and unextractably bound fractions, but the degree of association was different than chlorpyrifos, and microbial activity appeared to affect the distribution of radioactivity.

Further research will have to be performed to determine why atrazine associates with matrix and humic substances in an unextractable way and chlorpyrifos does not. In nature, dead plant material is decomposed to humus by microorganisms during catabolic and anabolic metabolic processes. Compounds such as amino acid, lipids, proteins, and carbohydrates are easily and quickly metabolized, however, lignocellulosic materials are resistant to microbial metabolism (Stevenson, 1982). As lignocellulosic material is metabolized by microorganisms, humic substances form (Sørensen, 1963). It has been demonstrated that composting plant material will result in humic substance formation in different total amounts and of variable compositions, depending on the type of plant material (Hammouda and Adams, 1986). During the decomposition of lignocellulosic materials, atrazine and any subsequent metabolites became strongly associated with the matrix fraction or incorporated into the humic substances during formation. Pesticide incorporation with humic substance formation can occur by proton-transfer and hydrogen bonding (Hayes, 1970), oxidative coupling (Berry and Boyd, 1985; Bollag, 1990), sorbative processes (Khan, 1990), and electron-transfer (Senesi (1992).

Sposito et al. (1996) proposed mechanisms for atrazine association with humic substances with varying acidity. The used optical and magnetic resonance spectral data to support a complexation mechanism between atrazine and humic substances, which is dependent on the basicity of the s-triazine molecule (e.g. atrazine) and the acidity of the humic substances. They postulate that proton-transfer mechanisms are favored in situations where the s-triazine has low basicity and the humic substances have higher amounts of acidic groups. Atrazine has a low pKa, which confers low basicity and, in general, fresh biobased materials used in composting have high amounts of fulvic acid and lower amounts of humic acids (Chen and Inbar, 1993). The other mechanism is electron transfer and is favored in situations with s-triazines that have high basicity (less electronegative components on s-triazine ring) and humic substances low total acidity. Fulvic acids have more acidic groups than humic substances (Stevenson, 1982). The electron-transfer seems unlikely for atrazine in this situation, but the proton-transfer mechanism is relevant to this situation and should be considered as a likely mechanism for atrazine complexation with humic substances. Atrazine and atrazine associated radioactivity should have been recovered in the extensive ethyl acetate and alkali
extractions if only hydrogen bonding, proton-transfer, or electron-transfer were the mechanism for atrazine and humic substance complexation. The fact that considerable radioactivity remained associated with the unextractable fractions suggests that substitution reactions or covalent bond formation was involved.

The ethyl and isopropyl side chains of atrazine can be cleaved by fungal populations, leaving the amino groups exposed (Levanon, 1993). It may be that atrazine is covalently bound to soil organic matter at the amino moieties of the triazine ring. It has been shown that phenolic amines can form covalent bonds with quinone compounds in coupling experiments with horseradish peroxidase, which would be similar to those reactions occurring humic substance formation (Berry and Boyd, 1985). However, both atrazine and chlorpyrifos have heterocyclic nitrogen, which should reduce the reactivity of the substituted secondary amines.

Haider et al. (1993) have shown through a series of experiments the mechanisms by which the fungicide anilizine, 4,6-dichloro-N-(2-chloro-phenyl)-1,3,5-triazine-2-amine, covalently binds to soil organic matter. They demonstrated that the chlorines at the triazine moiety exchanged with hydroxy groups of the soil organic matter (e.g. humic acids), forming esters or ethers. It is interesting to note from Chapter V that atrazine degraded quickly in a similar bioreactor system, whereas hydroxyatrazine did not quickly dissipate. The mechanism proposed by Haider et al. (1993) would explain the fate of atrazine in a lignocellulosic based bioreactor. Atrazine can dissipate by either direct hydrolysis to hydroxyatrazine or by covalent bond formation with oxygen containing functional groups in soil organic matter. Once the hydroxyatrazine is formed, the likelihood of covalent bonding with the hydroxyl group in hydroxyatrazine would be greatly reduced due to the stability of a keto-enol form. Any further covalent bonding with hydroxyatrazine and soil organic matter should occur at the amino moiety attached to the triazine ring.

Formation of a keto-enol form was most likely the reason that the chlorpyrifos hydrolytic product TCP did not form covalent bonds with the soil organic matter. Chlorpyrifos and TCP did not form unextractably bound residues or covalent bonds with soil organic matter, which is likely due to steric hinderance of the reaction leading to the ester or ether formation. Also, there are differences between the chlorine-carbon moieties of a triazine and pyridinyl ring structure with respect to nucleophilic attack by oxygen containing groups in soil organic matter.
Detoxification of pesticides in this waste disposal system does not necessarily have to end with mineralization or metabolite formation. Dissipation evidently includes volatilization as observed with chlorpyrifos. Ultimately, mineralization represents the most complete disposal process. However, increasing mineralization may not always be feasible in the pesticide waste disposal system described here because, depending on the pesticide's chemical nature, mineralization dramatically decreases as bound residues increase. Increased formation of bound residues and increased association of atrazine with the fulvic acid fraction should be considered a route of detoxification.

Kearney (1976), Frietag et al. (1984), Berry and Boyd (1985), and Bollag (1991). I believe that once a toxic chemical compound is unextractably associated to soil organic matter, the original chemical identity is obliterated and no longer represents an immediate threat to the environment. However, Khan (1982, 1991), as well as Bollag (1991), have stated that even though a "toxic" compound may essentially be removed as a threat to the environment, it can slowly be released from the complex and return to some form of the original species. This release process does not occur to any great extent or rate, thus any release into the environment is slow and most likely negligible (Kearney, 1976; Bollag, 1991). Khan (1991) believes that the original species, or degradates, are still available to plant uptake and movement into the environment, but admits that this process is slower than if the original species were free or lightly associated with a matrix. The statement made by Kearney (1976) concerning bound pesticide residues is very appropriate for the detoxification of atrazine in the Virginia Tech pesticide waste disposal system: "it can be argued that the binding of soil residues represents the most effective and safest method of decontamination by rendering the molecule innocuous and allowing slow degradation in the bound state to products that pose no short-term or long-term problems."

On the other hand, efforts to improve the detoxification of chlorpyrifos in the bioreactors should be focused on improving the hydrolysis of chlorpyrifos. Increasing the pH during isolation and incubation may increase the rate of hydrolysis. It has been demonstrated in Chapter V that amending the bioreactors with corn meal increased hydrolysis. It seems likely that the detoxification of chlorpyrifos could be improved by culturing a species or consortium of microorganisms capable of hydrolyzing chlorpyrifos.

In conclusion, the pesticide waste disposal system tested appeared to be a more effective means to dispose of atrazine than chlorpyrifos. DT$_{50}$ values of approximately 4 for atrazine was rapid, considering that the initial concentrations was 5000 mg/kg. The dissipation of chlorpyrifos from an initial concentration of 1000 mg/kg was slower with a
$DT_{50}$ value of approximately 10 weeks. Evidently, certain SSF conditions will need to be improved in the future, if the intention is to dispose of chlorpyrifos in a timely manner. The keyword to consider is timely. Chlorpyrifos will eventually degrade to concentrations that are acceptable, but the time and management of the system required may be counterproductive. Therefore, the approaches to improve detoxification of atrazine and chlorpyrifos in the SSF system described here should be different, considering the differences in the fate of each pesticide.
5 CONCLUSIONS

The research presented in this Dissertation was performed with the sole purpose to study the various factors involved in the pesticide waste disposal system under development at Virginia Tech, and use the results to optimize the detoxification of the pesticide waste. The pesticide waste disposal system that is illustrated in Figure 1 (Chapter 1, Introduction) involves two phases: (1) an isolation phase, and (2) a detoxification phase. The primary isolation phase involves sorption or containment onto various lignocellulosic matrices, such as peat moss and steam-exploded wood fibers. The second phase involves taking the lignocellulosic matrices with the isolated pesticide phase to bioreactors designed to detoxify the pesticide waste through a combination of abiotic and biotic processes. The bioreactors used to detoxify pesticides are based on composting technology. It was hypothesized for this research that greater biological activity will increase pesticide detoxification.

Pesticide-laden wastewater or rinsate constitutes a disposal problem for the pesticide user. Brosten (1988) estimates that upwards of 50,000 gallons of pesticide-laden rinse water can be generated in a growing season by a large agricultural operation. Most pesticide users may not generate this much pesticide-laden wastewater, but the concern of what to do with the hazardous waste remains. It is the aqueous waste generated from rinsate and wastewater that the initial phase of the Virginia Tech pesticide waste disposal system is being developed to remediate. Lignocellulosic matrices are used as sorbents to remove the majority of the pesticide from the aqueous phase.

Pesticides formulated as emulsifiable concentrates (EC) constitute nearly 75% of the pesticide used in the U.S. (Ware, 1994). It was determined in Project I that the emulsion formed by using an EC is a barrier for pesticide sorption. An emulsion may still retain its integrity even when diluted as with rinse water. Therefore, it is important to remove the emulsion barrier to sorption.

The EC examined was Dursban® 4E made to approximately 5000 μg chlorpyrifos/mL, which represents a typical concentration encountered as pesticide waste. The appearance of the initial Dursban® 4E solution was cloudy and white. The appearance of the solution became less cloudy and precipitated material settled to the bottom of the vial by simply allowing it to settle for 18 hours. The concentration of chlorpyrifos in solution after the settling period was 1391 μg/mL, which translated to a 67% reduction of the original amount of chlorpyrifos in solution. The remaining emulsion
after the settling period was stable and approximated the hydrophilic lipophilic balance (HLB) of Dursban® 4E.

Demulsification increased the overall amounts of chlorpyrifos that was sorbed. Demulsification was achieved with the addition of Ca(OH)₂ to the emulsion. It was also found that demulsification could be achieved by the sorbents without Ca(OH)₂ at sorbent amounts of 1 g/10 mL. Only the granular rubber was capable of demulsification at amounts < 1 g/10 mL. Demulsification was also achieved by passing the emulsion through a column of granular rubber and steam-exploded wood (SEW). Filtering decreased the concentration of chlorpyrifos in solution by removing the lignocellulosic matrix particles, and the associated chlorpyrifos, left suspended in solution after the demulsification step.

The demulsification treatment using Ca(OH)₂ followed by filtering was the most effective treatment for all sorbents for chlorpyrifos sorption. In solutions that had Ca(OH)₂ present and were filtered (DF), the sorbents AC, R, and SEW were significantly similar in their ability to decrease the aqueous concentration of chlorpyrifos (P > 0.05). The aqueous concentration of chlorpyrifos for AC, R, and SEW ranged from 2.2 ± 1.2 μg/mL (or 99.95%; AC at 1.0 g) to 259 ± 25 μg/mL (or 94% sorption; SEW at 0.1 g). The results from Project II and Mullins et al. (1992, 1993) indicate that steam-exploded wood (SEW) and peat moss (PM) in the initial batch sorption step (demulsified and filtered) can remove 94 to above 99% of the pesticides tested.

Some of the more water soluble pesticides, such as metolachlor, can be difficult to remove from the aqueous phase in the batch sorption step as Mullins et al. (1992, 1993) reported. They were able to increase metolachlor sorption by adding vegetable oil to the SEW sorbent. Adding the vegetable oil to the sorbent also increased chlorpyrifos sorption, resulting in 0.1 μg/g chlorpyrifos from an initial concentration of approximately 4000 μg/g chlorpyrifos formulated as Dursban® 4E (Mullins et al., 1993). It is important to consider that the bulk of most pesticides are isolated onto the lignocellulosic matrices during the batch phase, which are then taken for detoxification. Substituting the lignocellulosic matrices with AC as the batch sorbent is not necessarily a good alternative in the pesticide waste disposal system for the following reasons: (1) chlorpyrifos may remain in solution at concentrations (2 μg/g) which warrant further clean-up measures, and would be similar to that required after a batch sorption with lignocellulosic materials; (2) it is logical to have the bulk of isolated pesticide on a matrix that supports biological activity in the bioreactors. The wastewater remaining after the initial batch sorption step can be passed through a secondary column of lignocellulosic materials and then tertiary
filters containing AC and/or specially designed sorbents (Figure 1, Introduction). The final effluent would be water that is safe to reuse in non-potable agricultural practices, such as making tank mixes of pesticide or for irrigation.

From the isolation stage of the Virginia Tech pesticide waste disposal system, lignocellulosic matrices containing the pesticide are placed in bioreactors where the pesticide is detoxified through various abiotic and biotic paths. Different pesticides will dissipate at different rates in compost bioreactors. In these studies, malathion quickly degraded in field composting bioreactors. The DT$_{50}$ values for malathion were approximately a week. Conversely, chlorpyrifos - also an organophosphate insecticide - degraded much slower than malathion under similar conditions, having DT$_{50}$ values between 7 and 19 weeks. Malathion is a very labile compound being hydrolyzed quickly in aqueous and soil environments (Devine, 1987). On the other hand, chlorpyrifos does not degrade rapidly in soil, and is chosen as a termicide because of its persistence in soil, having DT$_{50}$ values between 116 and 1576 days applied at 1000 µg/g (Racke et al., 1993).

It was concluded in field experiments that atrazine and chlorpyrifos dissipation in compost bioreactors was increased with increased biological activity. The reasons for the enhanced pesticide dissipation with increased temperatures are twofold. Rates of abiotically and biotically mediated reactions increase with increased temperatures in compost environments. Therefore, if a pesticide is metabolized by biologically mediated reactions, then it stands to reason that more biological activity will increase the metabolism. Figure 47 illustrates the various paths of atrazine and chlorpyrifos dissipation. There are some similarities of fate between the two pesticides, as well as some differences, which will be discussed.

Volatilization can be a significant path of pesticide dissipation, though volatilization did not appear to be a significant path for atrazine dissipation. In the radiolabeled studies (Project VI), only 0.2% of the radioactivity originally applied as $^{14}$C - atrazine was recovered in the volatile traps over 16 weeks at 40°C. Volatilization did appear to be a major dissipation path for chlorpyrifos in compost bioreactors. Approximately 18% of radioactivity originally applied as $^{14}$C-chlorpyrifos was recovered in the volatile traps (Table 24, Project VI). It was believed that chlorpyrifos would volatilize out of the compost above 40°C, but in the actual situation, less than 0.003% of
Figure 47 Fate of Atrazine and Chlorpyrifos in Compost
the approximate 23 g of chlorpyrifos applied to field compost units (with temperatures reaching 65 °C) was recovered in volatile traps. Nicholson (1981) in his M.S. thesis on the fate of diazinon in compost suggested that volatilized diazinon may condense on the outer cooler compost matrix. A sample was taken from the uppermost part of the compost overlying the center of compost matrix where the chlorpyrifos was originally applied (Figure 19, Project III) and approximately 120 µg/g chlorpyrifos was detected. Apparently, volatilized chlorpyrifos condensed on the outer cooler compost matrix. It is difficult to conclude what percentage of the chlorpyrifos applied did volatilize and condense, because the sample taken was from one small area in the outer part of the compost. Further sampling of the overlying compost was not performed.

Depending on the vapor pressure of the pesticide and conditions, upwards of 90% loss by volatilization has been reported in field applications (Taylor, 1978). Trifluralin is considered to be a volatile pesticide (15 mPa, 20 °C) (Taylor and Spencer, 1990) and a 26% loss by volatilization was reported after 9 days and 90% loss by volatilization after 35 days (White et al. 1977).

It is likely that chlorpyrifos volatilization was occurring in the compost, since temperatures in the compost reached approximately 65 °C early in the experiment (Figure 27). The volatilization of chlorpyrifos may actually be greater in compost that has chlorpyrifos added to the entire matrix, rather than the relatively small portion (approx. 15 L) of compost matrix that was kept in the center of the untreated compost in the volatility experiment in Project III. The concentration of volatile pesticides in the effluent air should be monitored during the detoxification phase as Mullins et al. (1989) did with compost pits containing diazinon. Volatilization is an acceptable path of pesticide dissipation in this disposal system, but it is important to ensure that levels do not exceed inhalation health standards.

Once atrazine and chlorpyrifos are placed into the compost environment they can become distributed in the compost in three basic situations, which are the vapor phase, dissolved in the matrix water, or sorbed to the solid matrix. There are distribution equilibria between the three basic situations. Since atrazine and chlorpyrifos are nonpolar pesticides, they will have a thermodynamic tendency to partition to the more nonpolar environments, such as the matrix. Even though the majority of atrazine and chlorpyrifos would be associated with matrices in some fashion, there would be a constant exchange with atrazine, chlorpyrifos, and their metabolites in the matrix water phase.
Atrazine and chlorpyrifos can degrade when they are associated with the matrix or in the matrix water phase through abiotically and biologically mediated reactions. The hydrolysis of atrazine to hydroxyatrazine (HYA) and the hydrolysis of chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP) are considered to be the major degradative pathways (Figure 47). Apparently, this was the case in the experiments performed here, because both HYA and TCP were found in relatively high concentrations from compost. In the one cubic meter field compost experiment (Project III), HYA and TCP concentrations were approximately 180 and 650 µg/g, respectively, after 16 weeks of incubation (Figure 28, Project III). Initial atrazine and chlorpyrifos concentrations were approximately 3487 and 7421 µg/g, respectively. Most researchers believe that the atrazine and chlorpyrifos hydrolysis reactions are predominantly abiotic (atrazine: Armstrong et al., 1967; chlorpyrifos: Chapman and Harris, 1980). However, Harris (1965) and Mandelbaum et al. (1993) suggest that atrazine hydrolysis can be enhanced by microbes. There are similar reports of microbes enhancing chlorpyrifos hydrolysis (Miles et al., 1983; Getzin and Rosefield, 1968). The hydrolysis of atrazine can be catalyzed by interactions with unionized carboxyl groups in the soil organic matter (Armstrong, 1967), which may have occurred in the compost due to its large amount of organic material. A similar hydrolytic catalysis has not been demonstrated for chlorpyrifos.

The HYA and TCP can then be further degraded and eventually mineralized as shown in Figure 47, however, their persistence in the compost studies performed here suggest that they do not readily degrade. It has been demonstrated that HYA is more strongly sorbed to organic material than atrazine, which may partially explain its persistence. There have not been similar reports for TCP, but it can be assumed that TCP becomes associated with organic material. There is variable information concerned with the availability of sorbed molecules to microbial attack. Some researchers believe that sorbed nonpolar compounds are not as available for microbial metabolism (Manilal and Alexander, 1991), while others have shown that sorption is not a barrier for microbial metabolism (Thomas and Alexander, 1987). Dissipation of HYA and TCP in the compost experiments appears to have been slower than the rates of hydrolysis that formed them, therefore, it appears that HYA and TCP are more recalcitrant to degradation than their parent pesticide compounds. The sorption of HYA and TCP to the compost matrices may reduce the ability of microbes to metabolize them.

There were differences between the fates atrazine and chlorpyrifos in compost. In the radiolabeled experiments (Project VI), practically all of the matrix-associated
radioactivity (MAR) in chlorpyrifos bioreactors was recovered in the organic solvent extract after 16 weeks incubation, whereas only 20 to 30% of the MAR was recovered in the organic solvent extract from the atrazine bioreactors. This suggests that chlorpyrifos hydrolyzes to TCP. Neither the chlorpyrifos or the TCP became associated with the alkali soluble and insoluble fractions, suggesting that the association was loosely sorbed and reversible. The structure of the TCP molecule may actually hinder being chemisorbed to the lignocellulosic material in the compost as decomposition and humification occur. TCP is most likely to be in its "keto" form rather than its "enol" form, which decreases the potential for oxidative coupling to the "enol" form. Additionally, there are three chlorines associated with the 2-pyridinol moiety, which should also hinder enzyme-mediated oxidative coupling. Chlorpyrifos in microbially active soils has been shown to be almost completely mineralized (Cink and Coats, 1993), which means TCP can be metabolized by microorganisms. Part of the reason why TCP degradation appears to be slow in compost could be a result of the higher initial concentration of chlorpyrifos and slow microbially mediated degradation. Contrary to other reports (Racke et al., 1988; Felsot and Pederson, 1991), the presence of chlorpyrifos and TCP did not appear to have a toxic effect on compost microbial activity, even at concentrations of approximately 7000 and 2000 μg/g, respectively. TCP toxicity to soil fungi may occur because the fungal populations are small in comparison to the fungal populations present in the compost. The more active microbial populations in compost may better absorb toxic effects due to the large biomass. Also, if certain microbial species are adversely affected in compost by TCP, other species (bacteria) may proliferate, making any determination of toxic effects by examining microbial activity difficult.

It appears that atrazine or its metabolites readily form bound residues. Upwards of 60 to 65% of the MAR in the atrazine bioreactors after 16 weeks was associated with the alkali-soluble and insoluble fractions. It is not clear what are the mechanisms of association with these two fractions. Physical interactions of atrazine and atrazine metabolites with the organic matter and humic substances do occur and there is some controversy as to what are the mechanisms. Atrazine association organic matter and involvement with humic substance formation can occur by proton-transfer and hydrogen bonding (Hayes, 1970), oxidative coupling (Berry and Boyd, 1985; Bollag, 1990), sorbative processes (Khan, 1990), and electron-transfer (Senesi (1992). The association of atrazine or its metabolites with the humic substance fraction was most likely through
covalent bond formation, since no further radioactivity was recovered after extracting the precipitated humic acid fraction with ethyl acetate.

It has been shown that dealkylation of s-triazines is exclusively a microbially mediated process (Kruger et al., 1993; Kaufman and Kearney, 1970). Triazine dealkylation does commonly occur in soil with de-ethylatrazine (DEA) being the most common dealkylated residue detected (Skipper an Volk, 1972), however, Kruger et al. (1993) suggested that the DEA and DIA may form at equal rates, but the lack of DIA residue may be due to its faster degradation, which they demonstrated. The heightened microbial activity in the compost probably increased the dealkylation of atrazine and its metabolites. It is interesting that DEA, DIA, didealkylatrazine (DAA), de-ethyl-hydroxyatrazine (DEHYA), and desisopropyl-hydroxyatrazine (DEHYA) were detected at concentration less than 3 μg/g in compost that had initially 4750 μg/g atrazine. In other studies, the dealkylated metabolites have been detected in soil at similar or higher concentrations than in the compost studied here (Kruger et al., 1993; Schiavon, 1988), but the initial concentration in the soil studies was approximately 5 μg/g.

I believe that the lack of the dealkylated metabolites in the compost and the fact that bound residues do form are strong points to support the concept that the dealkylated metabolites are involved with the formation of the alkali soluble and insoluble fractions through a yet unknown mechanism. Other workers (Kruger et al., 1993) analyzed for the chlorinated dealkylated and hydroxylated dealkylated atrazine metabolites in soil and amounts recovered were also a small comparison to the percentage of radioactivity associated with the bound fraction. Atrazine and HYA were recovered in from Project V, suggesting that they are loosely associated with the compost matrix and not part of the unextractably bound fractions (alkali soluble and insoluble). The mechanism of the chemisorption of atrazine metabolites would involve an exposed triazine amino moiety and some oxygenated moiety of the humic substance or organic matter during humification. The role of microorganism is also unclear. From this study (Project VI), it appeared that heightened microbial activity did not increase bound formation, rather a difference in distribution. Cook et al. (1995) demonstrated that higher temperature bioreactors resulted in more unextractable bound residues. From Winkleman and Klaine (1991 a, b), sterilized soil controls exhibited similar percentages of unextractably bound radioactivity as in bioactive soil samples.

One possibility of atrazine/humic substance association may involve glutathione-S-transferase (GST) mediated coupling in the solid state fermentation environment
(Kearney, 1976). However, this is questionable too, since the GST is of biological origin and bound residue formation was apparent in sterilized soil (Winkleman and Klaine, 1991 a, b).

Another possible chemisorption mechanism would involve a carbonyl moiety or quinone molecule in the compost. The dealkylated triazine represents a primary amine, which oxidatively couples with a carbonyl carbon or ortho carbon in a quinone molecule (Figure 47). Both carbonyl carbons and quinones are present during organic matter decomposition and humification (Stevenson, 1982). In compost, the humification process is greatly exaggerated in comparison to the process in soil, therefore it appears that the formation of covalently bound residues from the dealkylated atrazine metabolites is a major dissipation path. Haider et al. (1993) demonstrated that a triazine called anilazine associated with humic substance hydroxyl groups at the halogenated moiety of the triazine ring by exchange of the halogen (in this case, chlorine too). They also concluded that covalent bonding of the secondary amino groups in anilazine with sulphydryl groups in the humic substance is unlikely. This mechanism is an interesting possibility because it occurs through abiotic means, which supports the observation by Winkleman and Klaine (1991 a, b) that unextractable bound residue formation occurred in sterile soil. Haider et al. (1993) also concluded that the mechanism of exchange occurs only with the chlorine on the triazine ring, and once it becomes hydroxylated through abiotic hydrolysis, which is a completely different reaction, the mechanism will not occur. This may be why so much HYA was observed in Project V. Once HYA is formed it does not appear to dissipate quickly (Project V). It would appear that HYA is not conducive for coupling with organic matter or humic substances, however, Winkleman and Klaine (1991 b) showed that HYA incubated in soil did form unextractably bound residue.

Two paths of atrazine dissipation may be occurring in soil and in a solid state fermentation environment: the direct hydrolysis of atrazine to HYA and coupling with carbonyl moieties of humic substances. This mechanism may explain why there was not much dealkylated metabolites found that retained the chlorine. It does not explain why hydroxylated and dealkylated atrazine metabolites are not found in quantity in this study or other studies. It would seem likely that the HYA would be dealkylated through biotic means quite readily and would be recovered in an analysis. It may be that these metabolites are coupled more readily and will not be found in quantity. Obviously, there is some mechanism responsible for unextractably bound residue formation in this system and
further research must be conducted to discern what mechanisms and interactions are responsible.

In the pesticide waste disposal system under development at Virginia Tech, the formation of bound residue through chemisorption should be considered a means of detoxification. It may not be as complete as mineralization, but the original compound loses its identity when it becomes covalently bound to other and possibly larger molecules. Even though Khan and Ivarson (1981) demonstrated that bound prometryn (s-triazine) could be released by further microbial degradation, they state that the process is slow. Bollag (1991) also contends that the bound residues can be released back into the environment, but the amounts are negligible over time. The dealkylated atrazine metabolites retain some phytotoxicity, therefore their destruction by bound residue formation is beneficial.

At some point it has to be determined by residue analysis if the spent compost can be discarded in the normal waste stream in a landfill or taken to be land applied. Because the resulting water from the initial phase of the pesticide waste disposal process and the aged or spent compost from the secondary phase constitute treated hazardous waste, the EPA requires a permit for their final disposal. To acquire a permit for disposal of treated hazardous waste, the petitioner must convince the EPA that the waste will not pose a threat to the environment or human health. Sampling the effluent water and the spent compost for pesticide residue would indicate if the concentrations are below the maximum contaminant levels (MCL) promulgated by the EPA as directed by the Safe Drinking Water Act (1986). It is likely the water will be clean enough to reuse for nonpotable agricultural uses, but it is equally likely that the spent compost will have some pesticide residue associated with it. Even with a 99% reduction in pesticide waste originally at 5,000 to 20,000 µg/g, there may residues at 10 to 100 µg/g in composted pesticide waste. The overall detoxification of pesticide waste in compost is good, but if the waste is to be land applied, it has to be determined if the pesticide remaining (or metabolites) will leach into the environment. If some degree of leaching is possible, it has to be determined what levels are acceptable. I think if the spent compost with the remaining pesticide waste is applied on agricultural land, where it would normally be used, and applied in such a manner as to essentially dilute any remaining pesticide, it is a safe and viable practice and should be done. In addition to eliminating pesticide waste, the soil organic matter will be improved on the land where the spent compost is applied, which is good for soil fertility. In addition to residue analyses, which will involve private or government laboratories, the
safety of landapplying spent compost can be further tested using the toxicity characteristic leaching procedure (TCLP) designed by the EPA (EPA, 1991) as Berry et al. (1992) did with spent compost containing carbofuran and atrazine residues. They found that neither pesticide in spent compost posed a toxicological or leaching threat.

There is an unfortunate aspect to what appears to be a good and safe termination of the pesticide waste. Licensed and skilled people, either at the private or government level, will have to be involved in ensuring that the pesticide waste is correctly treated during the isolation and detoxification stages of the pesticide waste disposal system, and in determining if the final waste products (i.e. water and spent compost) can be safely used in agricultural practices.

If it appears that land application is not a possibility because the EPA cannot be convinced of its safety, then it may be best to develop the initial sorption stage, and forgo developing the detoxification stage. If the detoxified waste would have to be incinerated or taken to a landfill anyway, it seems logical to bypass the detoxification stage, and take the isolated pesticide waste directly to an incineration or landfill facility.

If it appears that land application or some other final disposal can be done in a cost efficient manner, then it is important to keep the compost bioreactors hot and healthy; the compost temperature should exceed 50°C, and it should be kept aerobic by maintaining good airflow (bulking agents and turning). Also, the importance of compost inhabiting invertebrates assisting with pesticide degradation needs to be considered. To take advantage of enhanced decomposition by compost invertebrates, a good cooling phase (1 to 2 weeks) should be allowed to occur in the compost which gives the invertebrates the opportunity to move in to the cooler regions of the compost. To maintain compost at temperatures near 50°C, the following measures should be performed. The volume of the compost should be approximately 1 m³. Bulking materials, such as peanut hulls, should be mixed in with the compost matrices. Nitrogen (e.g. chicken manure) has to be added to the compost for microbial growth - adding nitrogen appeared to be one of the most important keys to high temperature in the compost tested here. The compost should be hydrated, but not to the point where air flow is restricted and oxygen concentrations decrease. Energy amendments, such as corn meal and vegetable oil will enhance microbial activity, and it was demonstrated in the studies presented here that pesticide dissipation is also enhanced. The compost should be mixed approximately once every two to three weeks. Two to three weeks appeared to be a long enough period of time for the compost to go through a thermophilic stage, followed by a cooling phase. Permitting the cooling
stage allows beneficial vertebrates to move into the compost where they enhance overall decomposition. Additionally, fungi and actinomycetes proliferate in the final cooling stage. Their role in decomposing vegetative material in the compost may make them an integral part of pesticide degradation. Finally, the compost unit needs to be constructed in a way that prevents unintentional contamination of the surrounding soil and groundwater. If the recommended compost practices are included in the management of compost bioreactors designed to detoxify pesticides, the best composting conditions should be achieved to mitigate the pesticide waste created.
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David attended Radford University in Radford, Virginia where he received a Bachelor of Science degree in 1984 in two majors, biology and chemistry. David received his Masters of Science degree in Entomology on 17 October. His Master of Science thesis was Flight Activity and Hemolymph Diacylglyceride Concentrations in Heliothis zea (Boddie) (Lepidoptera: Noctuidae). David was accepted as a Ph.D. student in Entomology at Virginia Tech in 1989. He was married to Jean Marie Madigan on 2 June 1990. He is currently a Candidate for a Ph.D. degree in Entomology at Virginia Polytechnic Institute & State University. David is currently employed with the AgrEvo USA Company, a Company of Hoechst and NQR-AM, located in Pikeville, North Carolina as a soil chemist in the Environmental Chemistry Department.

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