

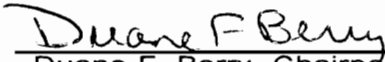
DEVELOPMENT OF SORPTION TECHNOLOGY FOR THE CLEANUP OF  
PESTICIDE CONTAMINATED WASTEWATER

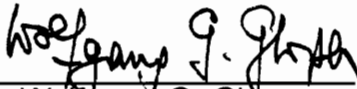
by

Hans P.L. Willems

Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and  
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Doctor of Philosophy  
in  
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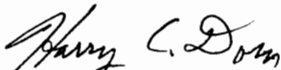
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# DEVELOPMENT OF SORPTION TECHNOLOGY FOR THE CLEANUP OF PESTICIDE CONTAMINATED WASTEWATER

Hans P.L. Willems

Dr. D.F. Berry, Chair

Crop and Soil Environmental Sciences

## ABSTRACT

The use of pesticides in agricultural production has led to the contamination of surface and groundwater. There is a need for simple, on site wastewater cleanup procedures to minimize pollution caused by spills and improper disposal of pesticide wastes by pesticide applicators and farmers. Sorption technology may prove a viable alternative to biological wastewater treatment. Properties of the ideal sorbent are (i) a high capacity for the contaminant, (ii) inexpensive and/or regenerable, and (iii) easy to dispose of after use. Our objective was to evaluate Filtrasorb 400 (activated carbon), Ambersorb adsorbents, and thiol derivatized cellulose beads for pesticide adsorption. Also, the use of solid state fermentation as a means of final disposal of pesticide sorbed onto a lignocellulosic matrix was investigated. Adsorptive capacities of Ambersorb adsorbent 572 for dicamba as Banvel 4L and metolachlor as Dual 8E under flow through conditions were three times higher than adsorptive capacities of Filtrasorb 400. Better performance of Ambersorb adsorbents under flow through conditions was likely the result of faster mass transfer kinetics caused by a more favorable pore size distribution in Ambersorb adsorbents. Ambersorb adsorbents are easily regenerable, with a slight loss of adsorptive capacity, using methanol. Thiol derivatized cellulose beads were synthesized using a novel method. Carbonyldiimidazole activated

cellulose beads were reacted with aminoethanethiol to yield thiol contents of up to 160  $\mu\text{moles/mL}$  beads. Sorption of metolachlor by ethanethiol-cellulose beads was primarily the result of chemisorption, although some physisorption may occur. Chemisorption of metolachlor by ethanethiol-cellulose beads is the result of a nucleophilic substitution reaction, immobilizing metolachlor. Chemisorption increased at elevated temperatures and pH. Sorptive capacity of ethanethiol-cellulose beads for metolachlor in batch studies was similar to Ambersorb adsorbent 572. Ethanethiol-cellulose beads may prove a useful sorbent for contaminants containing electrophilic centers, such as atrazine, simazine, alachlor, and metolachlor. Solid state fermentation of carbofuran in a peat matrix showed that a large amount of carbofuran residues became incorporated into organic matter. The formation of carbofuran-7-phenol was necessary for incorporation to occur. Carbofuran-7-phenol likely was incorporated through peroxidase catalyzed oxidative coupling reactions. Solid state fermentation of contaminants that can undergo oxidative coupling, may prove an effective detoxification method.

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## **CHAPTER I**

### Introduction

The increased use of pesticides has led to the contamination of groundwater, surface waters, and soil, and constitutes a threat to drinking water quality (Huang and Frink, 1989; Exner, 1990; Roux et al., 1991). Metolachlor, alachlor, and atrazine are the top three herbicides used in the USA, totaling 170 million lb active ingredient per year and make up 40% of all herbicides used in the USA (Gianessi and Puffer, 1991). All those herbicides have been detected in groundwater, surface waters, and groundwater recharge structures. Some triazines and acetanilides accumulate in soil profiles and constitute a potential for leaching to groundwater (Huang and Frink, 1989).

Risks are especially high for atrazine, metolachlor, and alachlor because of their persistence in the environment (Cavalier et al., 1991). A large scale groundwater monitoring study for metolachlor indicated that high pollution levels of groundwater could in most cases be traced back to point sources (spills related to metolachlor storage, mixing, and handling) (Roux et al., 1991). Point source pollution could easily be reduced by on-site wastewater treatment, combined with improved handling procedures.

To date, the majority of treatment procedures are either too complicated or too expensive to be used by small scale pesticide applicators and farmers (Norwood, 1990). The Pesticide Waste Disposal Group at Virginia Tech, whose members are located in the departments of Crop and Soil Environmental Sciences, Entomology, Biochemistry and Anaerobic Microbiology, and Biological

Systems Engineering, is an interdisciplinary research team, working to develop pesticide disposal technology. The goal of this group is to develop an efficient, low cost, environmentally sound, on-site pesticide waste disposal system. It is envisioned that small scale farm operators and pesticide applicators will use this system to reduce environmental contamination.

The basic pesticide disposal strategy evaluated in this work, involves the adsorption of pesticides on biologically-based sorbent materials (e.g., peat moss, steam-exploded wood) followed by *in situ* microbial degradation of adsorbed pesticide residues during solid state fermentation (SSF). Regenerable synthetic sorbents can be used as an alternative to biologically based sorbents. When considering using a sorbent regeneration strategy, the desorbed pesticide can merely be reused or subjected to microbial degradation in a lignocellulosic matrix (SSF) (Fig. 1).

The use of sorbent regeneration as a strategy was investigated in the second chapter. Two synthetic adsorbents, activated carbon (Filtrisorb 400) and a polystyrene-divinylbenzene based sorbent (Ambersorb adsorbent) were evaluated for metolachlor (as Dual 8E) and dicamba (as Banvel 4L) adsorption and subsequent regeneration.

In the third chapter, the synthesis of a biologically based adsorbent, namely beaded ethanethiol-cellulose, is described. The introduction of a nucleophilic group on beaded cellulose, in this case SH, may increase the

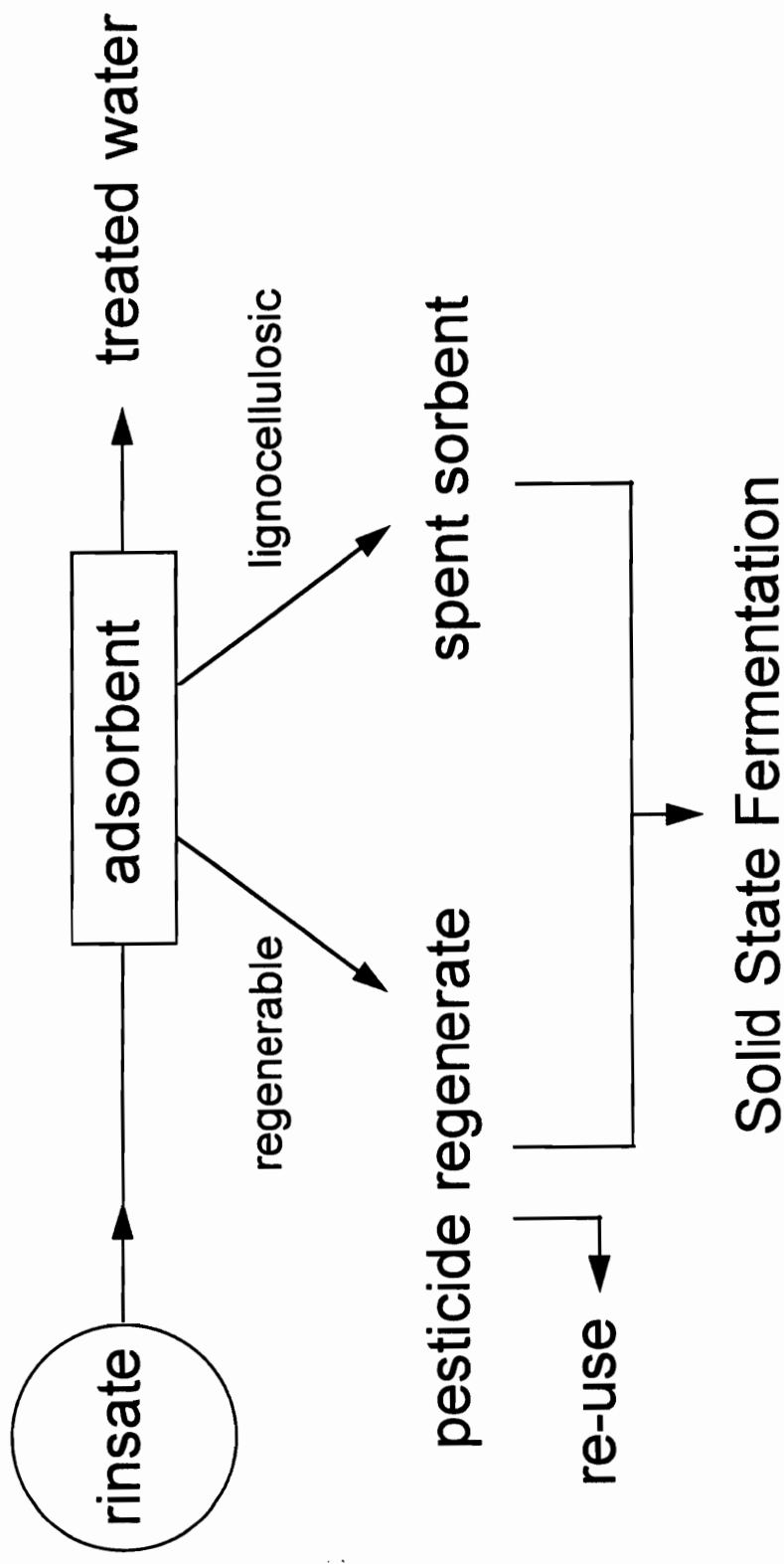


Figure 1. Pesticide-waste treatment system.



affinity of the adsorbent for electrophilic pesticides such as the chloroacetanilides (metolachlor, alachlor), triazines (atrazine, simazine), thiocarbamates, organophosphates, and aromatic compounds with e-withdrawing groups (2,4-D).

Subsequently (chapter IV), the ethanethiol-cellulose beads were tested for metolachlor adsorption under static and flow through conditions. The effects of temperature, pH, and flow rate on adsorption were evaluated.

As mentioned above, the final stage of the disposal strategy involves the microbial degradation of the adsorbed or regenerated pesticide in a lignocellulosic matrix (SSF). The fate of carbofuran during solid state fermentation in a peat moss and in a peat:peanut hulls:steam-exploded wood matrix was investigated and the subject of chapter V.

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## **CHAPTER II**

### **Evaluation of Synthetic Adsorbents for Clean-up of Herbicide Laden Wastewater**

## ABSTRACT

Small- and large-scale farm operators and applicators urgently need user-friendly and cost-effective on-site methods to treat dilute-formulated herbicide-laden wastewater (i.e., rinsate). In this study, we investigated solvent-regenerable synthetic adsorbents, Ambersorb® 563 adsorbent, Ambersorb 572 adsorbent, and Ambersorb 575 adsorbent, as alternatives to activated carbon (Filtrisorb® 400) for cleanup of water containing Dual 8E (a.i., metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide]) or Banvel 4L (a.i., dicamba [3,6-dichloro-2-methoxybenzoic acid]). Batch-type adsorption experiments were conducted to compare adsorptive capacities of the various adsorbents. Adsorption maxima, obtained from adsorption isotherms, indicated that of the Ambersorb adsorbents tested, Ambersorb 572 adsorbent generally exhibited the highest adsorptive capacities for the herbicides tested and was comparable to Filtrisorb 400. Solvent (methanol) regenerability studies demonstrated that herbicide was readily recovered from Ambersorb 572 adsorbent and Filtrisorb 400. In general, metolachlor was more readily recoverable than dicamba. In-fixed bed column studies, Ambersorb 572 adsorbent exhibited twice the adsorptive capacity of Filtrisorb 400. Fixed-bed columns were more effective in removing metolachlor from herbicide-containing water regardless of the adsorbent used. Multicycling of Ambersorb 572

adsorbent resulted in 30% loss of adsorptive capacity for dicamba as Banvel 4L and no loss of adsorptive capacity for metolachlor as Dual 8E. Working capacity was reached after three adsorption-regeneration cycles. Fixed-bed columns packed with solvent-regenerable adsorbents may prove useful as part of an on-site wastewater disposal system.

## INTRODUCTION

Improper disposal of formulated pesticide solutions, including rinsate, is a potential hazard to soil, surface waters, and groundwater. Proper disposal of rinsate, which can contain anywhere from 30 to 2000 mg L<sup>-1</sup> of herbicide (Toller and Flaim, 1988), is especially troublesome to small-scale farm operators because they are infrequent users and often use a diverse variety of pesticide products. Currently, the majority of the methods available for pesticide wastewater treatment are either too costly or involve complicated procedures or equipment (Norwood, 1990). A useful on-site rinsate cleanup technology should be safe, user-friendly, economical, and effective for a broad range of formulated herbicides. Such a system could also be useful for large-scale applicators, and could be integrated in cleanup procedures for large, contaminated waterbodies. One of the key factors in such a system would be the use of an adsorbent material that is either regenerable or readily disposed of when spent.

Mullins et al. (1992a) proposed a biologically-based system for treatment of pesticide-laden wastewater that included batch adsorption using lignocellulosic materials such as peat moss or steam-exploded wood fiber, followed by separation (filtration) and finally, degradation of the sorbed pesticide. In this system, the lignocellulosic sorbent material can be recycled as a sorbent after it is subjected to a pesticide degradation cycle. In an on-site

operation, it may be desirable to use fixed-bed adsorption columns, rather than batch-type adsorption systems, to decontaminate pesticide-laden wastewater because of the ease of operation and better effluent quality, though maximum adsorbent capacity is more readily attained in a batch-type system.

The purpose of this study was to evaluate the use of fixed-bed adsorption columns packed with a solvent-regenerable adsorbent as a means to decontaminate herbicide-containing water. Performance of three Ambersorb adsorbent materials was compared with Filtrasorb 400, a commonly used universal adsorbent. The common herbicides, metolachlor and dicamba (with water solubilities of 530 and 6500 mg L<sup>-1</sup>, respectively), were used in this study.

## MATERIALS AND METHODS

**Chemicals and adsorbent materials.** Ambersorb 563 adsorbent, Ambersorb 572 adsorbent, and Ambersorb 575 adsorbent were provided by Rohm and Haas Co., Philadelphia, Penn. Filtrasorb 400 was purchased from Calgon Carbon Corp., Huntington, West Virginia. Selected properties of the adsorbents are shown in Table 1 (Rohm and Haas, 1990; Parker, 1992). Technical grade (TG) metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide], 97% purity, and dicamba [3,6-dichloro-2-methoxybenzoic acid], 98.7% purity, were purchased from Chem Service, West

**Table 1. Selected properties of adsorbent materials.**

Adsorbent	Surface Area (m <sup>2</sup> /g)	Porosity <sup>a</sup> (mL/g)			Pore ratio	
		Micro < 2 nm	Meso 2-50 nm	Macro > 50 nm	Macro + Meso	Micro
Ambersorb 563	550	0.23	0.14	0.23	0.62	0.62
Ambersorb 572	1100	0.41	0.19	0.24	0.95	0.95
Ambersorb 575	800	0.32	0.20	0.20	0.8	0.8
Filtrisorb 400	1100	0.48	0.09	0.04	3.69	3.69

<sup>a</sup> N<sub>2</sub> adsorption



Chester, Penn. Formulated herbicides Dual 8E and Banvel 4L were provided by Ciba-Geigy Corp. and Sandoz Corp., respectively. Dual 8E (active ingredient (a.i.) 86% metolachlor) is an emulsifiable concentrate, while Banvel 4L (a.i. 40% dicamba) is a soluble concentrate. All herbicide solutions prepared were true solutions, with the exception of Dual 8E preparations which were emulsions.

**Adsorption kinetics.** Thirty milliliters of either emulsified Dual 8E or Banvel 4L solution ( $3500 \text{ mg L}^{-1}$  a.i.) were added to 250-mL Erlenmeyer flasks containing one gram of a selected adsorbent. While the intended initial herbicide concentration target was  $3500 \text{ mg L}^{-1}$  a.i. for both herbicides, the actual measured concentrations were 4160 and  $3000 \text{ mg L}^{-1}$  a.i. for Dual 8E and Banvel 4L, respectively. Flasks were agitated (115 RPM) in a rotary incubation shaker at  $25^{\circ}\text{C}$ . Contents of the flasks were sampled periodically, and the concentration of herbicide in the aqueous phase was determined by high-performance liquid chromatography (HPLC). Adsorption kinetics were evaluated by plotting the amount of herbicide adsorbed versus time.

**Adsorption isotherms.** Batch-type adsorption studies were conducted for all herbicide-adsorbent combinations by adding 30 mL of herbicide solution ( $250 \text{ mg L}^{-1}$  for TG herbicides and 250 and  $3500 \text{ mg L}^{-1}$  a.i. for formulated herbicides) to 250-mL Erlenmeyer flasks containing either 0.01g, 0.05g, 0.1g, 0.25g, 0.5g, 0.75g, or 1g of adsorbent. Flasks were agitated (115 RPM) for 24 hours in a rotary incubation shaker at  $25^{\circ}\text{C}$ . The amount of herbicide adsorbed,

$S_e$  ( $\text{mg g}^{-1}$ ), was determined using Eq. 1.

$$S_e = \frac{(C_i - C_e)v}{1000w} \quad [1]$$

where  $C_i$  and  $C_e$  are initial and equilibrium herbicide concentrations ( $\text{mg L}^{-1}$ ), respectively,  $v$  is volume (mL) of herbicide solution, and  $w$  is weight (g) of adsorbent. Sorption isotherms were obtained by plotting  $S_e$  versus  $C_e$ .

Adsorption isotherms for formulated herbicide were constructed using data sets from both loading rates (i.e. 250 and 3500  $\text{mg L}^{-1}$ ).

**Adsorbent regeneration.** Regeneration experiments were conducted to evaluate the potential for re-utilization of the adsorbents. Prior to regeneration, 1 g of adsorbent material was contacted with 30 mL of either 250 or 3500  $\text{mg L}^{-1}$  (a.i.) Dual 8E emulsions or Banvel 4L solution in 250-mL Erlenmeyer flasks. Flasks were agitated (115 RPM) for 24 hours in a rotary incubation shaker at 25°C. Equilibrium herbicide concentrations were determined using HPLC, and the amount of herbicide adsorbed was calculated as described above. Following the adsorption phase, adsorbent materials were transferred to a column (8 cm long x 1 cm i.d.) and regenerated by infiltrating 100 mL of methanol under saturated flow conditions at a flow rate of 1  $\text{mL min}^{-1}$ . The methanol rinsate was collected in 10 mL fractions, and the herbicide concentration was determined by HPLC. Herbicide recovery was calculated on the basis of the amount of herbicide initially adsorbed. The adsorption-

regeneration cycle was repeated three times.

**Fixed-bed column studies.** A series of three fixed-bed adsorption columns received either Dual 8E or Banvel 4L (1500 mg L<sup>-1</sup> a.i.) at a flow rate of 47 mL min<sup>-1</sup> (equivalent to a hydraulic flow rate of 9.57 mL min<sup>-1</sup> cm<sup>-2</sup>, or a flow rate of 23 bedvolumes (BV) per hour through the first column). The columns, (25 cm long x 2.5 cm i.d.), were packed with either Ambersorb 572 adsorbent or Filtrasorb 400. Herbicide solutions were continuously stirred during delivery to the columns. Because Dual 8E is formulated as an emulsifiable concentrate, the concentration of influent was continuously monitored. Effluent was sampled periodically, following passage through each column, and the herbicide concentration was determined by HPLC. Column operations were stopped when effluent concentrations from the third column in the series reached 30 mg L<sup>-1</sup> (i.e., specified breakthrough concentration), which represents a 98% removal efficiency. Following breakthrough, the columns packed with Ambersorb 572 adsorbents were regenerated by infiltration with 1 L of methanol at a flow rate of 2 BV hr<sup>-1</sup>. Herbicide concentration was determined by HPLC. Methanol regenerant was removed by flushing the columns with distilled water (20 BVs). A total of three and four adsorption-regeneration cycles were conducted for columns receiving Banvel 4L and Dual 8E, respectively. Breakthrough curves were modeled by fitting the data to Eq. 2.

where  $C$  is the effluent herbicide concentration (mg cm<sup>-3</sup>),  $C_i$  is influent herbicide

$$\frac{C}{C_i} = \frac{1}{1+e^{a+bL}} \quad [2]$$

concentration ( $\text{mg cm}^{-3}$ ),  $a$  and  $b$  are constants, and  $L$  is throughput volume (L) or calculated using Eq. 3, derived by Bohart and Adams (1920).

$$\frac{C}{C_i} = \frac{1}{1+e^{K\left(\frac{N_o D}{V} - Ct\right)}} \quad [3]$$

where  $C$  and  $C_i$  are as in Eq. 2,  $K$  is a rate constant ( $\text{cm}^3 \text{mg}^{-1} \text{min}^{-1}$ ),  $N_o$  is maximum solid-phase concentration of herbicide ( $\text{mg cm}^{-3}$ ),  $V$  is linear flow rate ( $\text{cm min}^{-1}$ ),  $D$  is bed depth (cm), and  $t$  is service time in minutes. As is common practice, the values for  $N_o$  and  $K$  in Eq. 3 are determined by plotting breakthrough time versus bed depth (Cloutier et al., 1985; Ramalho, 1983; Hutchins, 1973). These plots, referred to as bed-depth-service-time (BDST) diagrams, can be described by the Bohart-Adams Eq. 3 solved for service time (Ramalho, 1983).

$$t = (N_o/C_i V)D - \ln[(C_i/C) - 1]/KC_i \quad [4]$$

From the slope and intercept of the BDST diagrams,  $N_o$  and  $K$  were calculated. The critical bed depth ( $D_o$ ) (i.e., the minimum bed depth required to meet breakthrough concentration at time zero) was calculated from Eq. 4 by setting  $t = 0$ .

$$D_o = (V/KN_o) \ln[(C_i/C) - 1] \quad [5]$$

**Analytical procedures.** Prior to HPLC analysis, samples were filtered through 0.2 $\mu$ m Gelman Acrodisc LC13 PVDF membrane filters. Aqueous-phase samples containing metolachlor were diluted with methanol (5:1) prior to filtration to prevent adsorption onto the filter membrane. Herbicide concentrations were determined using an HPLC system (Isco Inc., Lincoln, Neb.) consisting of a model 2350 pump and a V<sup>4</sup> variable wavelength detector. Metolachlor samples were chromatographed on a 15-cm Supelcosil LC-18-DB (5- $\mu$ m-particle size) column. The mobile phase consisting of methanol:H<sub>2</sub>O (80:20, v/v) was delivered at a flow rate of 1 mL min<sup>-1</sup>. Dicamba separation was accomplished using a 25-cm Supelcosil LC-18-DB (5- $\mu$ m-particle-size) column. The mobile phase acetonitrile:methanol:H<sub>2</sub>O:acetic acid (42.2:20:34.5:3.3, v/v) was delivered at a flow rate of 1 mL min<sup>-1</sup>. Metolachlor and dicamba were quantified by the external standards method at wave lengths of 220 and 271 nm, respectively.

## RESULTS AND DISCUSSION

**Adsorption kinetics.** Results of the metolachlor as Dual 8E ( $C_i = 3500$  mg L<sup>-1</sup>) adsorption study are shown in Fig. 1. Within the first hour of incubation, more than 95% of the metolachlor was removed from solution. The amount of metolachlor adsorbed after one hour was minimal. For dicamba as Banvel 4L,

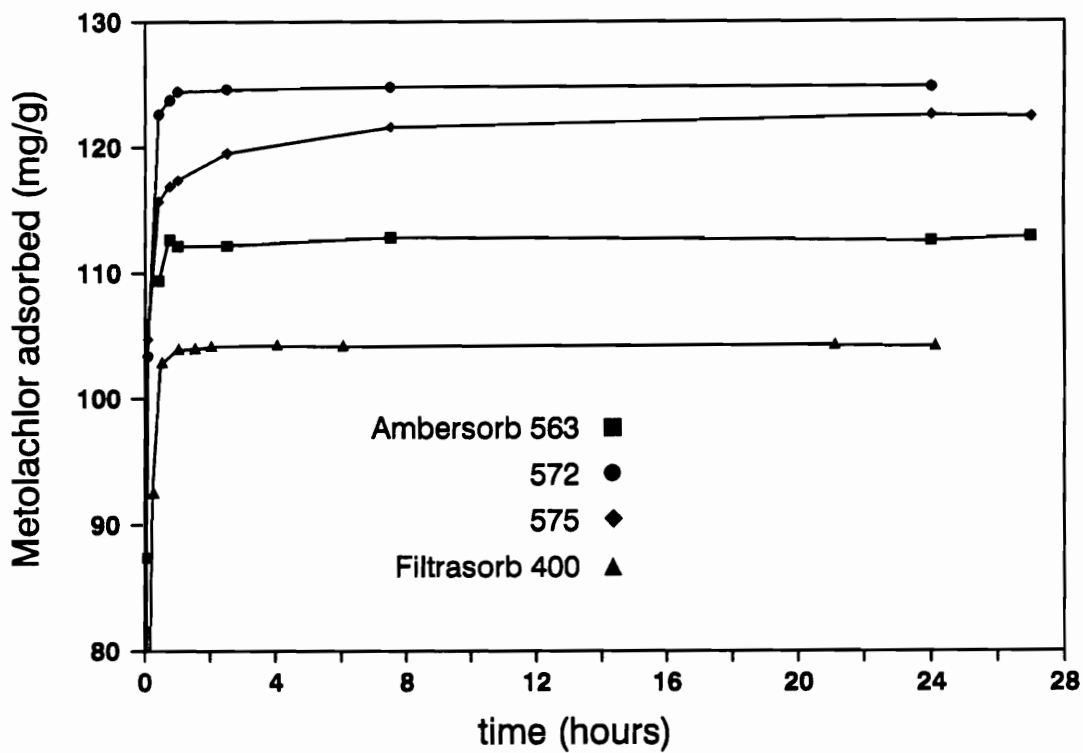


Figure 1. Adsorption kinetics of metolachlor as Dual 8E onto different test adsorbents.

we observed that more than 95% of the dicamba was adsorbed within 5 hours, with no significant amount of dicamba being adsorbed beyond that time period (data not shown). These results established that equilibrium conditions prevailed for all herbicide-adsorbent combinations within 24 hours.

**Adsorption isotherms.** Adsorption isotherms for adsorption of dicamba as Banvel 4L and metolachlor as Dual 8E onto Ambersorb 572 adsorbent are shown in Fig. 2. In all cases, a steep initial increase in the amount of herbicide adsorbed was followed by a plateau. At higher equilibrium concentrations, an additional increase in the amount of adsorbed herbicide was sometimes observed. The amount of herbicide adsorbed at the beginning of the plateau was taken as adsorption maximum ( $m$ ) and was used to compare the different adsorbents. The  $m$  values, determined by visual observation, are reported in Table 2.

Based on  $m$  values, Ambersorb 563 adsorbent exhibited the lowest adsorptive capacity for the herbicides tested. We suspect that the adsorptive capacity of Ambersorb 563 adsorbent was primarily a function of surface area and micropore volume (Table 1), though differences in the surface characteristics of this adsorbent when compared to the others was probably a contributing factor. Surface property differences between Filtrasorb 400 and Ambersorb 572 adsorbent were likely responsible for the large difference in their ability to adsorb technical-grade metolachlor (Table 2), given that these

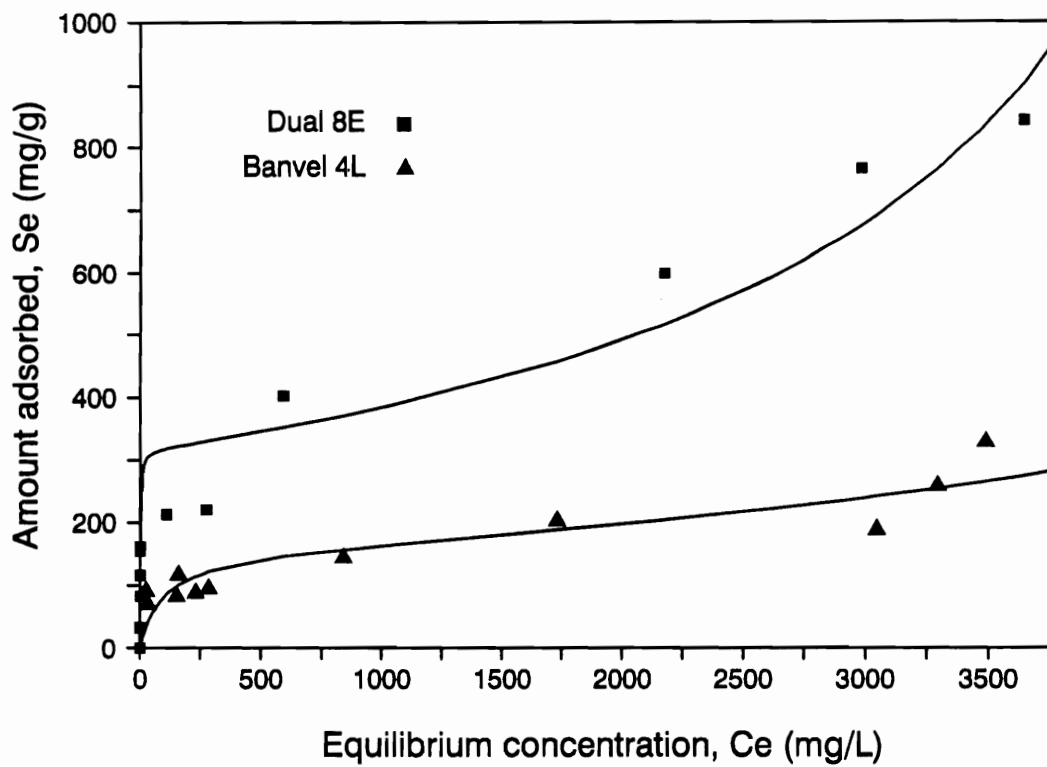


Figure 2. Adsorption isotherms of metolachlor as Dual 8E and dicamba as Banvel 4L onto Ambersorb 572 adsorbent.



**Table 2. Adsorption maxima for tested herbicide-adsorbent combinations.**

	Adsorption maxima <sup>1</sup> (mg/g)			
	Dual 8E	Banvel 4L	Metolachlor (TG)	Dicamba (TG)
Ambersorb 563	200	45	40	110
Ambersorb 572	375	125	180	180
Ambersorb 575	420	120	120	180
Filtrisorb 400	350	105	400	160

<sup>1</sup> m value

two adsorbent materials had equivalent surface areas (Table 1). Based on water adsorption isotherms (used as a measure of hydrophobicity), the surface of Ambersorb adsorbent was determined to be more hydrophobic than Calgon activated carbon (Rohm and Haas, 1990). The most hydrophobic adsorbent tested was Ambersorb 563 adsorbent, which was followed by Ambersorb 575 adsorbent, Ambersorb 572 adsorbent, and then Calgon activated carbon. Overall, the results show that Filtrasorb 400, Ambersorb 572 adsorbent, and Ambersorb 575 adsorbent had the largest capacities to adsorb metolachlor and dicamba (TG and formulated). Ambersorb 563 adsorbent was clearly the least effective adsorbent.

The  $m$  value was greatly influenced by the presence of adjuvants in formulated herbicides. In the case involving dicamba, the presence of adjuvants reduced the adsorptive capacity for dicamba. It is likely that the adjuvants competed with the dicamba molecules for adsorption sites. Unlike the situation involving dicamba, adsorptive capacities for metolachlor were larger for the formulated herbicide for all the Ambersorb adsorbents tested. This observation is not easily explained because at the high loading rate (Dual 8E, 3500 mg L<sup>-1</sup> a.i.) there are three phases that need to be considered including an aqueous phase, micellular phase, and a solid phase. Micelle adsorption, multilayering, and phase separation at the solid surface could result in high adsorptive capacities.

**Adsorbent regeneration.** Solvent regenerable adsorbents could prove useful in the development of an on-site wastewater cleanup process. On-site regeneration of herbicide-laden adsorbent would reduce costs and increase convenience. Typically, activated carbon is regenerated by use of a high-temperature furnace (800-900°C). Amborsorb adsorbents are reportedly regenerable using either steam, hot inert gases, or solvents such as methanol and acetone (Rohm and Haas, 1990). Methanol was used in this investigation.

The amount of herbicide recovered during regeneration usually increased with progressive adsorption-regeneration cycles (Table 3 and 4). This observation was most obvious at the lower herbicide concentrations ( $C_i = 250 \text{ mg L}^{-1}$ ). While there were some interesting trends, it was not possible to discern, with a high degree of confidence, those factors responsible for controlling herbicide recovery. Recoverability of herbicides was undoubtedly influenced by differences in attractive forces between the surface and sorbate, adsorbent porosity differences, the solubilizing capability of the regeneration solvent, and the presence of adjuvants.

Recovery of metolachlor (as Dual 8E), appeared to be related to micropore volume. In this case, mass transfer kinetics could be the major factor controlling recovery. It is also reasonable to conclude that the solubilizing capability of the regeneration solvent (i.e., methanol) was not adequate for dicamba at the low-concentration treatment level. A methanol-salt water mixture

**Table 3. Regeneration of Dicamba as Banvel 4L from different adsorbents.**

Adsorbent	Cycle	C <sub>i</sub> (mg/L)	C <sub>e</sub> (mg/L)	adsorbed (mg/g)	% recovery
Ambersorb 563	1	250	0.90	7.48	36.67
	2	250	2.60	7.43	54.33
	3	250	5.20	7.35	69.68
Ambersorb 572	1	3051	1462.30	47.66	64.08
	2	3051	2020.80	30.90	92.82
	3	3051	1969.40	32.45	87.52
Ambersorb 575	1	250	ND	7.51	5.23
	2	250	ND	7.51	6.81
	3	250	0.51	7.49	20.71
Filtrisorb 400	1	3051	13.50	91.12	86.53
	2	3051	147.90	87.09	90.03
	3	3051	205.10	85.37	94.12
Ambersorb 575	1	250	ND	7.51	5.73
	2	250	ND	7.51	10.41
	3	250	0.61	7.49	23.08
Filtrisorb 400	1	3051	73.50	89.32	72.94
	2	3051	305.70	82.36	87.05
	3	3051	440.00	78.33	97.03
Filtrisorb 400	1	250	ND	7.51	2.81
	2	250	1.57	7.46	10.40
	3	250	0.78	7.49	18.20
Ambersorb 572	1	3051	168.89	86.46	53.90
	2	3051	657.26	71.81	100.50
	3	3051	701.61	70.48	101.29

C<sub>i</sub> = initial concentration, C<sub>e</sub> = equilibrium concentration and ND = not detected

**Table 4. Regeneration of Metolachlor as Dual 8E from different adsorbents.**

Adsorbent	Cycle	C <sub>i</sub> (mg/L)	C <sub>e</sub> (mg/L)	adsorbed (mg/g)	% recovery
Ambersorb 563	1	269	ND	8.07	80.58
	2	269	ND	8.07	88.66
	3	269	0.41	8.06	107.28
	1	3785	401.96	101.49	88.07
	2	3785	426.50	100.76	101.33
	3	3785	457.87	99.81	93.30
Ambersorb 572	1	269	ND	8.07	46.72
	2	269	ND	8.07	62.03
	3	269	0.50	8.06	83.07
	1	3785	4.21	113.42	71.87
	2	3785	2.47	113.48	86.66
	3	3785	10.70	113.23	100.72
Ambersorb 575	1	269	ND	8.07	62.13
	2	269	0.22	8.06	90.50
	3	269	ND	8.07	96.17
	1	3785	241.12	106.32	83.96
	2	3785	325.01	103.80	93.05
	3	3785	382.54	102.07	95.70
Filtrisorb 400	1	269	1.62	8.02	54.28
	2	269	1.60	8.02	75.82
	3	269	1.82	8.02	86.40
	1	3785	5.90	113.37	80.61
	2	3785	1.60	113.50	84.05
	3	3785	14.60	113.11	94.85

C<sub>i</sub> = initial concentration, C<sub>e</sub> = equilibrium concentration and ND = not detected

as the regeneration solvent might increase recovery of dicamba significantly, given that dicamba is a very water-soluble herbicide, which exists in its anionic form ( $pK_a \cong 2.0$ ) (Cheng, 1990).

The working capacity of the adsorbent can be defined as the capacity that is maintained over a large number of adsorption-regeneration cycles. In some cases (Beccari et al., 1977; Martin and Ng, 1985), a drop in adsorptive capacity is observed during the first few adsorption-regeneration cycles. Once working capacity has been reached, the adsorptive capacity of the adsorbents should stay constant during further adsorption-regeneration cycles, and all adsorbed herbicide should be recovered. Therefore, we believe, selection of the most effective adsorbent should be based on working capacity. For the most part, our results established that percent recovery after the third cycle approached 100%. We concluded, based on the results for the third cycle, that for adsorption of metolachlor as Dual 8E (3500 mg L<sup>-1</sup>) and dicamba as Banvel 4L (3500 mg L<sup>-1</sup>), Ambersorb 572 adsorbent was the most effective.

**Fixed-bed column studies.** Although batch adsorption isotherm studies are useful in evaluating adsorptive capacities of various adsorbents, results from batch systems cannot be relied upon to accurately predict performance of a sorbent under continuous-flow application conditions. Because Ambersorb 572 adsorbent performed well in both batch adsorption and solvent regenerability studies, it was selected for use in the continuous-flow fixed-bed

column studies. Filtrasorb 400 was also used in fixed-bed column studies for comparison purposes.

The metolachlor as Dual 8E breakthrough curves for the Ambersorb-adsorbent packed columns are shown in Fig. 3. The first column breakthrough curve for Filtrasorb 400 fed with Dual 8E is included in Fig. 3 to allow easy comparison with Ambersorb 572 adsorbent. The second and third column breakthrough curves for Filtrasorb 400 were not included in Fig. 3 for the purposes of clarity. Similar breakthrough curves were observed for the other herbicide-adsorbent combinations. Solid lines were obtained by fitting the data to Eq. 2. The dotted lines represent the calculated breakthrough curve using Eq. 3 derived by Bohart and Adams (1920). In the graph,  $t$  values were replaced by throughput volumes,  $L$ .

BDST diagrams for Banvel 4L adsorption onto Ambersorb 572 adsorbent are shown in Fig. 4. The results for  $N_o$ ,  $K$ , and  $D_o$  are reported in Table 5. It is noteworthy that breakthrough curves generated using Eq. 3, with  $N_o$  and  $K$  obtained from BDST curves, did not fit the observed breakthrough curves adequately (dotted lines, Fig. 3). Because  $N_o$  is directly proportional to the area to the left of the breakthrough curves, in our case, the BDST-approach overestimated  $N_o$ . Using the curve-fit approach,  $N_o$  ( $N_o$  values between brackets, Table 5) values (as determined by the Riemann sum approximation method) were consistently lower, thus, caution should be exercised when using

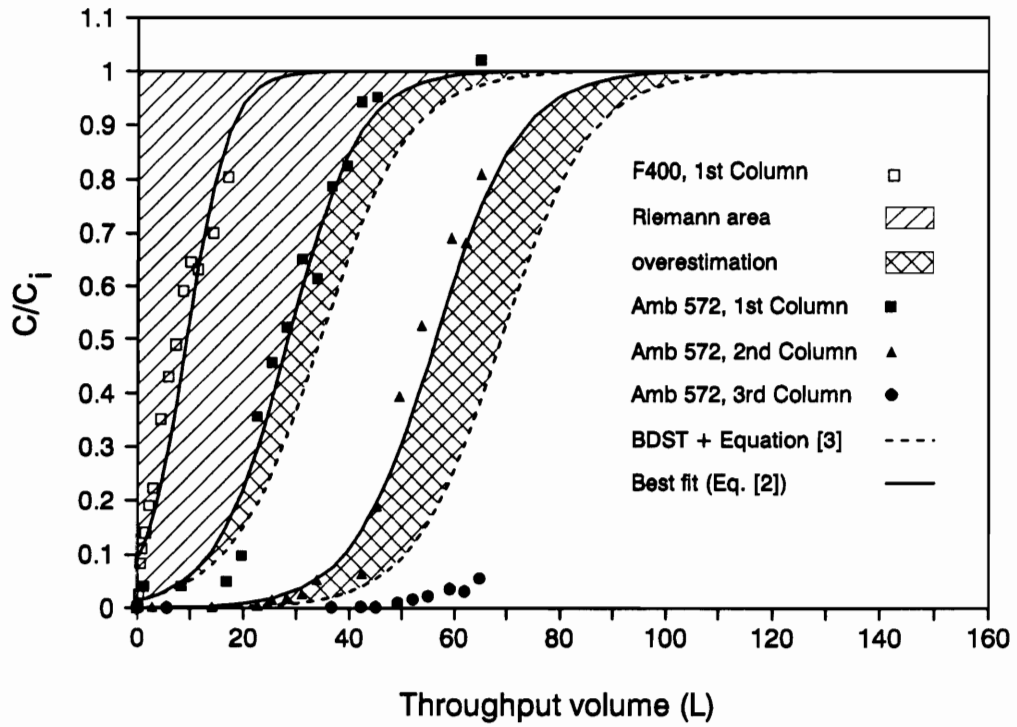


Figure 3. Breakthrough curves for Ambersorb 572 adsorbent and Filtrasorb 400 fed with Dual 8E.



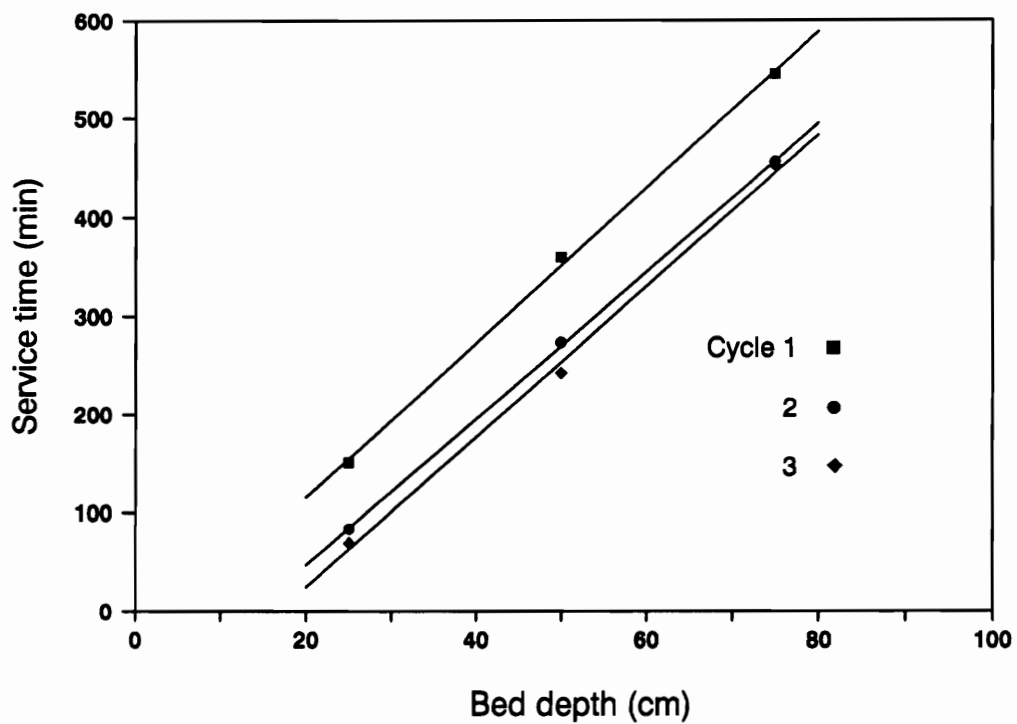


Figure 4. BDST-curve for Banvel 4L adsorption onto Ambersorb 572 adsorbent ( $r^2$  ranged from 0.98 to 0.99).

**Table 5. Calculated values for  $N_0$ ,  $D_0$ , and  $K$  from BDST diagrams.**

Adsorbent	BDST parameters	Banvel 4L				Dual 8E			
		-----Cycle-----				-----Cycle-----			
		1	2	3	1	2	3	4	
Ambersorb 572	$D_0$ (cm)	5.33	13.7	16.74	23.54	24.22	24.22	14.02	
	$N_0$ (mg/cm <sup>3</sup> )	113.03	107.08	109.70	317.03	359.79	291.98	(279.9)	
	$K$ (cm <sup>3</sup> /mg min)	(88.5)	(61.0)	(61.3)	(260.1)	0.005	0.004	0.009	
Filtrisorb 400	$D_0$ (cm)	0.062	0.025	0.020	44.19	-	-	-	
	$N_0$ (mg/cm <sup>3</sup> )	23.71	-	-	148.69	-	-	-	
	$K$ (cm <sup>3</sup> /mg min)	61.03	-	-	(97.4)	-	-	-	
		(36.1)	-	-	0.006	-	-	-	
		0.026	-	-	-	-	-	-	

$N_0$  values in parentheses were determined by the Riemann sum approximation method.

the BDST approach to determine adsorptive capacities of carbonaceous adsorbents used in cleanup of formulated herbicide-laden wastewater.

Based on  $N_0$  values as determined by the curve-fit method, the adsorptive capacity (first cycle) of Filtrasorb 400 for metolachlor as Dual 8E and dicamba as Banvel 4L was 62 and 59% lower, respectively, than the adsorptive capacity of Ambersorb 572 adsorbent. This large difference in adsorptive capacity was not observed in the adsorption isotherm studies, where Ambersorb 572 adsorbent and Filtrasorb 400 were approximately equally effective. We believe that mass transfer limitation caused this difference. Mass transfer of the herbicides to the surface and into the micropores of the adsorbents has no effect on the batch adsorption isotherm. However, for fixed-bed column studies, adsorption is dependent on mass transfer kinetics and, therefore, is different from adsorption in batch-type reactors. The ease with which herbicide molecules diffuse into micropores determines in part column performance. We believe that Ambersorb 572 adsorbent has more favorable mass transfer kinetics than Filtrasorb 400. We contend that mass transfer is better facilitated in Ambersorb 572 adsorbent, because of the large micropore volume in combination with sufficient meso- and macropores (Table 1).

Both Ambersorb 572 adsorbent and Filtrasorb 400 performed best for adsorption of metolachlor as Dual 8E. Adsorptive capacity (first cycle) for dicamba as Banvel 4L was 66% and 63% lower for Ambersorb 572 adsorbent

and Filtrasorb 400, respectively. The difference between adsorption of metolachlor as Dual 8E and dicamba as Banvel 4L might be caused by the difference in solubilities of metolachlor and dicamba. The solubility of metolachlor is  $530 \text{ mg L}^{-1}$ , while the solubility of dicamba is  $6500 \text{ mg L}^{-1}$ . Therefore, dicamba has a greater tendency to stay in the aqueous phase, compared to metolachlor. We believe that the difference in formulation, i.e. Dual 8E versus Banvel 4L, is also an important factor. Banvel 4L contains 60% adjuvants, while Dual 8E only contains 14% adjuvants, so the competitive effect of adjuvants for the same adsorption sites is expected to be much larger for Banvel 4L than for Dual 8E.

Herbicide regeneration following the first adsorption cycle resulted in a recovery of 89% of adsorbed metolachlor as Dual 8E, and 53% of adsorbed dicamba as Banvel 4L. Comparison of adsorptive capacities, of the first and last cycle for Ambersorb 572 adsorbent, showed a reduction in adsorptive capacity of 31 and 0% for Banvel 4L and Dual 8E, respectively. Critical bed depth ( $D_c$ ) increased by a factor of 3 from the first to the third cycle for Banvel 4L and remained constant for Dual 8E. From the data, we reasonably could assume that working capacity was reached after three adsorption-regeneration cycles.

Complete on-site treatment of the wastewater would require that the pesticide regenerate be chemically decomposed or degraded in an environmentally responsible manner at the location where it is generated. It

may be possible to degrade the pesticide-laden methanol regenerate in bioreactors (Mullins et al., 1992b). This possibility would need to be investigated thoroughly prior to field testing.

## CONCLUSIONS

Our results indicate that Ambersorb 572 adsorbent was superior to Filtrasorb 400 in fixed-bed adsorption columns because of its greater adsorptive capacity and good regenerability characteristic. Regardless of the adsorbent used, fixed-bed columns were more effective in cleanup of metolachlor as Dual 8E containing water compared to dicamba as Banvel 4L containing water. Further research should be directed at improving the recovery of adsorbed pesticide to facilitate the recycling/regenerability of adsorbent materials. Also, the effect of linear flow rate on column performance should be investigated (optimization).

## ACKNOWLEDGMENT

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## **CHAPTER III**

Derivatization of Beaded Cellulose: Introduction of Sulfhydryl Groups



## ABSTRACT

Incorporation of xenobiotics into organic matter, oxidative coupling of xenobiotics, and glutathione conjugation are naturally occurring detoxification mechanisms. These mechanisms of detoxification involve covalent bond formation between the xenobiotic and a solid matrix (organic matter), or a biomolecule (soluble humic material, glutathione). Immobilization, through covalent bond formation, of pesticide residues onto solid supports may prove a useful strategy for the cleanup of pesticide residue containing waters. The solid support of choice in this research was beaded cellulose and the pesticide of interest was metolachlor. Reaction of metolachlor with aminobutyric acid and mercaptopropionic acid indicated that metolachlor was reactive towards SH groups, but not towards NH<sub>2</sub> groups. The reactivity increased with temperature and pH. Subsequently, five different reaction schemes (carbonyldiimidazole-S protection, oxothiazolidine, bis-oxirane, carboxymethylation, and carbonyldiimidazole pathway) were evaluated to introduce SH functionality onto cellulose beads. The carbonyldiimidazole-S protection and oxothiazolidine pathway were not successful. Carboxymethylation method and bis-oxirane method resulted in an SH content of 1 and 8 μmoles SH groups per mL of cellulose beads, respectively. The carbonyldiimidazole pathway was the most successful. Under the given conditions, a concentration of 100 μmoles SH per

mL of beads was obtained.

## INTRODUCTION

Detoxification of xenobiotics can be accomplished through immobilization by naturally occurring processes including incorporation into organic matter, and glutathione conjugation. Incorporation of xenobiotics (e.g., pesticides) into soil humic substances involves, in most cases, the formation of one or more covalent bonds between xenobiotic and constituents of the humus fraction. Whether incorporation detoxifies the xenobiotics depends primarily on the irreversible character of the covalent bond. It is generally accepted that xenobiotics can be incorporated into soil humus during humic substance synthesis (Bollag and Loll, 1983). The extent of incorporation depends on the chemical characteristics of the xenobiotic, and various soil properties such as temperature, moisture, organic matter content, pH, and microbial activity, to mention a few. Among the compounds that are most susceptible to incorporation are phenolic compounds and aromatic amines. In this way, all xenobiotics that can be biologically or chemically transformed to a phenolic or anilinic intermediate are susceptible to incorporation. The oxidative coupling reactions are catalyzed by peroxidases and laccases. These enzymes are believed to operate through free radical (or possibly quinone) type intermediates

(Figure 1). The rather nonspecific mechanism of these enzymes provides for a

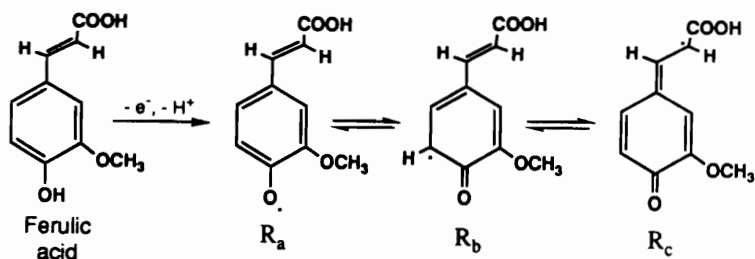


Figure 1. Formation of radical intermediates (Tatsumi et al. 1994).

coupling reaction that is applicable to a wide range of compounds.

Because it is difficult to distinguish, *in situ*, whether xenobiotics are incorporated by means of covalent bonds or adsorbed by means of physical forces (van der Waals forces), often times investigators refer to non extractable xenobiotic compounds simply as bound residues. As pointed out by Bollag and Loll (1983), the amount of bound residue reported in the literature is strongly dependent upon the extraction procedure. Thus, the fact that a compound is unextractable does not imply, *per se*, that it is incorporated by means of a covalent bond. Evidence of the occurrence of covalent bond formation has been obtained by several authors (Berry and Boyd, 1985; Tatsumi et al., 1994). Tatsumi et al. (1994) have demonstrated that peroxidase catalyzed reactions between phenolic and anilinic compounds and ferulic acid, a humic substance precursor, result in the incorporation of the xenobiotic into larger molecules

resembling humic substances (Figure 2).

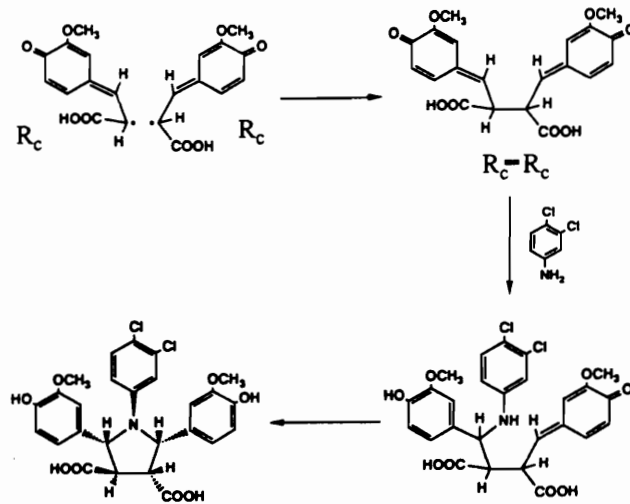


Figure 2. Coupling between ferulic acid and 3,4-dichloroaniline (Tatsumi et al. 1994).

From an environmental standpoint, the most important question is whether or not incorporated residues represent a hazard to the environment. In the process of soil organic matter turnover which is a slow process, some bound residues may be released and become available for plant uptake or leaching (Khan, 1980; Still et al., 1980). Bollag and Bollag (1990) stated that, to date, all available data indicate that release of bound residues is minimal, suggesting that once bound to humus, xenobiotics are unlikely to adversely affect the environment.

A different detoxification mechanism involving covalent bond formation, commonly encountered in nature, is glutathione conjugation (Meister and

Anderson, 1983). This mechanism involves the formation of a covalent bond between glutathione and a xenobiotic (Figure 3). The conjugate can be further

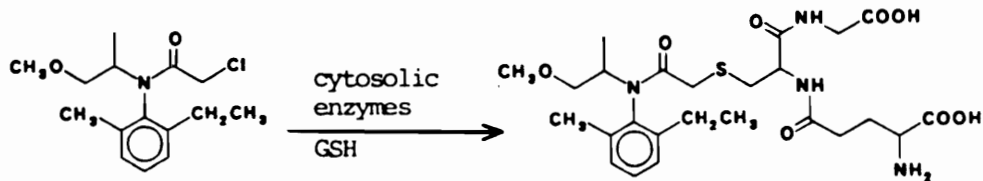


Figure 3. Reaction between glutathione and metolachlor (Feng and Wratten, 1989).

metabolized through the mercapturic acid pathway (in mammals and insects) (Feng and Patanella, 1988; Habig et al., 1974), or is a nontoxic end product by itself (in plants) (Anderson and Gronwald, 1991). The reaction is commonly catalyzed by glutathione S-transferase. These proteins are found in mammals (Mannervik and Danielson, 1988), insects (Grant and Matsumura, 1989), plants (Anderson and Gronwald, 1991), and bacteria (Fahey et al., 1978). Xenobiotics that can be detoxified in this way need to have an electrophilic group, which reacts with the nucleophilic -SH group of glutathione. Some commonly used pesticides, susceptible to glutathione conjugation are the triazine herbicides; atrazine and simazine, the chloroacetanilides; alachlor and metolachlor, and the thiocarbamates; thiobencarb and pebulate.

Anderson and Gronwald (1991) observed that resistance of maize and sorghum to atrazine can be explained based on high levels of glutathione and

glutathione S-transferase (GST). Soil transformation of acetochlor, a chloroacetanilide, through glutathione conjugation was reported by Feng (1991). The reported half-lives of acetochlor and its glutathione conjugate in soil were 7 days and 30 minutes, respectively. Glutathione and other soluble thiols, are present in a broad range of bacteria, however, the occurrence of a number of bacteria that lack glutathione indicates that glutathione is not specifically required for essential processes. Apontoweil and Berends (1975) observed that glutathione negative bacterial mutants were more susceptible to a wide range of chemicals than their glutathione positive parents. These findings indicate the importance of glutathione in detoxification mechanisms.

Glutathione conjugation can occur without GST catalysis, driven only by the inherent reactivity of the nucleophilic SH group of glutathione towards an electrophilic center containing xenobiotic. Also, it is likely that other thiol containing biomolecules, or biomolecules containing different nucleophilic moieties can be active in detoxification.

Manipulation of covalent bond forming detoxification processes, mentioned in the above discussion, can lead to applications for the cleanup of contaminated soils and water. For example, soil peroxidases can be induced to couple organic contaminants to soil organic matter by addition of  $H_2O_2$  (Berry et al., 1985). One can also take advantage of the oxidative coupling process during SSF to bind organic contaminants, such as pesticides, to humic

substances. Incorporation of pesticide residues can be optimized by carefully selecting the organic matter source and reaction conditions (temperature, moisture, and pH). Another strategy, based on the same principle, was reported by Klibanov (1983). Klibanov demonstrated the feasibility of peroxidase-catalyzed removal of phenols from wastewaters. He showed that treatment with horseradish peroxidase and  $H_2O_2$  precipitated 97 to 99% of the phenolic compounds as large polymers.

A different method, based on the glutathione conjugation mechanism, is to attach nucleophilic groups to a solid support, which can react with compounds containing electrophilic centers, thus immobilizing them. Immobilization of biomolecules such as enzymes and substrates, on a solid matrix is common practice in affinity chromatography, and finds applications in a wide range of disciplines. Immobilization of toxic chemicals containing electrophilic moieties (e.g., certain pesticides) may be a way to detoxify contaminated water.

A wide range of solid supports, such as agarose, cellulose (White, 1985), dextran, porous particulate alumina, and controlled pore glass are used in affinity chromatography. In order to function as a highly efficient adsorbent, the ideal matrix should have several properties: beads should have a loose porous network; beads should be uniform in porosity and size, spherical and rigid; derivatives must be easy to form; beads should be able to withstand moderate

pressures; and beads should be stable for some time. (Dean et al. 1985).

Disposal of the support material following pesticide immobilization is also a matter of concern. In this respect, biodegradable supports (cellulose, agarose, dextran) might be preferable. In my research, biodegradable cellulose beads were used as a support for immobilization of the chloroacetanilide herbicide metolachlor.

Cellulose is a polymer of  $\beta$ -1,4 linked glucose units (Figure 4).

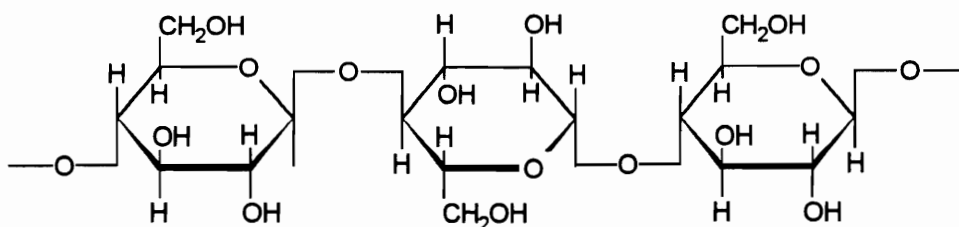


Figure 4. Structure of cellulose.

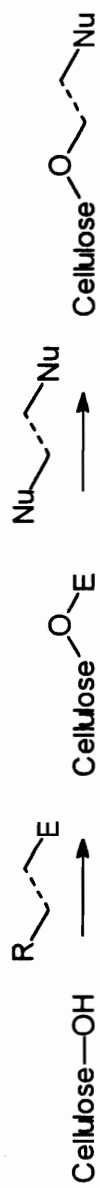
Beaded cellulose has a porous structure, making the reactive sites relatively accessible. The spherical particles are rigid because of the occurrence of microcrystalline regions interconnected with amorphous material (Dean et al. 1985) resulting in a favorable mechanical stability which is important in column operations. Theoretically, each glucose unit contains 3 free hydroxyl groups, available for derivatization. In reality, a large part of the hydroxyl groups cannot



be used for derivatization because they are involved in H-bonding or are not accessible because they are located in microcrystalline regions (Krässig, 1985).

The first step in the derivatization of a solid matrix for affinity chromatography is activation of the matrix. Commonly, activation involves the introduction of an electrophilic center on the matrix. When immobilization of a nucleophilic compound is the final objective, all that needs to be done is to react the activated matrix with the desired ligand under the appropriate conditions. However, when the final objective is the immobilization of an electrophilic center containing compound, such as metolachlor, a second step, introducing a strong nucleophile on the matrix, is required. This can be done by reacting the activated matrix with a cross-linking reagent. The cross-linking reagent typically contains two nucleophilic moieties. One nucleophile reacts with the activated bead, while the other nucleophile is available for immobilization of the electrophilic center containing compound (Figure 5).

Numerous procedures for the activation of carbohydrate polymers have been developed. One of the most widely used activation procedures is the cyanogen bromide (CNBr) method. CNBr introduces, through a reaction with vicinal diols, a highly reactive isourea function (Kaster et al., 1993; Peng et al., 1987; Dean et al., 1985). This reaction is carried out in an aqueous medium. Several activation methods introduce epoxide groups on the beads. Epoxide groups can be introduced through a reaction of the beads with a bis-oxirane



E: electrophilic group      Nu: nucleophilic group

Figure 5. Generic derivatization of cellulose beads

(e.g., 1,4-butanediol diglycidoxy ether) or through epichlorohydrin (1-chloro-2,3-epoxypropane) activation (Axen et al., 1975; Gemeiner and Zemek, 1981; Gemeiner and Benes, 1983; and Boeden et al., 1991). By using bis-oxiranes or chloro-epoxides of different lengths the spacer length can be varied (Axen et al., 1975). The epoxide activation methods are also carried out in aqueous medium. Reaction of beads with chloroacetic acid in aqueous NaOH produces carboxymethylated beads (Boeden et al., 1991; and Dean et al., 1985). Activation of agarose through reaction with *s*-norbornene 2-3-dicarboximido carbonchloridate under anhydrous reaction conditions has been described by Buettner et al. (1989). Carbohydrate beads can also be activated through reaction with various carbonyldiimidazoles (e.g., 1,1'-carbonyl diimidazole) (Dean et al., 1985). Because carbonyldiimidazoles can react with water, anhydrous reaction conditions are required for this reaction. Sodium periodate oxidation of beaded cellulose results in the formation of activated cellulose dialdehyde beads (Boeden et al., 1991). Other activation procedures include triazine activation (Benes et al., 1991), tosylchloride activation (Peng et al., 1987), polyisocyanate reactions (Dean et al., 1985), and isothiocyanate activation (Gemeiner et al., 1977). The activated beads can be used to immobilize biomolecules containing nucleophilic groups, or can be further derivatized (e.g., introduction of sulfhydryl or amine functions) through reaction with a ligand of choice.

The goal of my research was to evaluate the strategy of pesticide immobilization on a solid matrix for purposes of decontaminating pesticide contaminated wastewater. The objective of this phase of my research was to derivatize beaded cellulose with a nucleophile that would chemisorb metolachlor, an electrophilic center containing herbicide. In order to accomplish this, I evaluated the reactivity of  $\text{NH}_2$  and  $\text{SH}$  towards metolachlor and determined the most effective means to derivatize the cellulose beads with the most appropriate functionality.

## MATERIALS AND METHODS.

### **Reaction of metolachlor with mercaptopropionic and aminobutyric acid.**

*Chemicals.* Technical grade (TG) metolachlor was purchased from Chem Service, West Chester, Penn. 3-Mercaptopropionic acid and 4-aminobutyric acid were obtained from Aldrich Chemical Co., Inc.

*Chemistry.* To 50 mL of a 100 mg L<sup>-1</sup> metolachlor solution, buffered (sodium tetraborate buffer) at a preset pH value, 154 µl mercaptopropionic acid or 181 mg aminobutyric acid was added in 125 mL Erlenmeyer flasks. The pH values for the reaction with mercaptopropionic acid were 7.5, 9, and 10. pH values for the reaction with aminobutyric acid were 8,9, and 10. The Erlenmeyer flasks were placed on an incubation shaker at 25 °C, 37.5 °C, and 50 °C and sampled at regular time intervals (15 min, 30 min, 45 min, 1, 1.5, 2, 3, and 4 h). Controls, in which mercaptopropionic acid and aminobutyric acid were omitted, were included. Metolachlor concentrations in the reaction mixtures were determined using an HPLC system (Isco Inc., Lincoln, NE) consisting of a model 2350 pump and V<sup>4</sup> variable wavelength detector. Samples were chromatographed on a 15-cm Supelcosil LC-18-DB (5-µm-particle size) column. The mobile phase consisting of methanol:H<sub>2</sub>O (80:20, v/v) was delivered at a flow rate of 1 mL min<sup>-1</sup>. Metolachlor was quantified by the

external standard method at wavelength of 220 nm. The ratio between nucleophile and metolachlor was 100:1, so that pseudo first order kinetics applied:

$$\frac{dC}{dt} = -kC$$

Where  $C$  is the concentration of metolachlor in mg/L and  $k$  is the pseudo first order reaction constant in  $\text{min}^{-1}$ . The reaction constant,  $k$ , is the slope of the plot  $\ln C_x - \ln C_o$  versus  $t_x - t_o$  (where  $C_x$  is concentration of metolachlor at time  $t_x$ , and  $C_o$  is initial metolachlor concentration at time  $t_o$ ).

#### **Derivatization of cellulose beads.**

*Chemicals.* 2-Aminoethanethiol hydrochloride, 2,2'-dipyridyl disulfide (2-aldrithiol), 1,1'-carbonyldiimidazole, sodium methoxide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1,4-butanediol diglycidyl ether, dithiothreitol (DTT), chloroacetic acid, sodium salt, tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and 2-mercaptopyridine were purchased from Aldrich Chemical Co., Inc. Ferrous sulfide and sodium borohydride were purchased from Fisher. Cellulose beads, prepared as described by Kaster et al. (1993), were kindly provided by Dr. W.G. Glasser (Department of Wood Science and Forest Products, Virginia Tech).

*Imidazole activated amine, thiolpyridine protected -SH.* (Figure 6). Under an N<sub>2</sub> atmosphere, 2.27 g aminoethanethiol hydrochloride, in 50 mL EtOH was slowly added to 5.28 g of 2-aldrithiol in 50 mL EtOH. Reaction mixture was held at 22 ± 1°C while stirring for 4.5 h. Upon addition of the aminoethanethiol the solution turned yellow instantaneously and a white precipitate formed before addition of aminoethanethiol hydrochloride was completed. At the end of the reaction the precipitate was removed by filtration and solublized in MeOH. Based on an NMR (Varian 400 MHz) spectra, the precipitate was identified as aminoethanethiol (Figure 7). The filtrate was concentrated in a rotavap, leaving a viscous yellowish liquid. The viscous liquid was dissolved in 15 mL chloroform and any precipitate was removed. Purification of the unknown compounds was achieved using a silica G column. The gradient solvent system used to elute the compounds from the column was 99:1 (v/v) CHCl<sub>3</sub>:MeOH until 2-aldrithiol was removed, then, 97:3 (v/v) until all the yellow color was eluted (released thiolpyridine), and finally 97:2:1 (v/v/v) CHCl<sub>3</sub>:MeOH:triethanolamine to elute compound **1**. Compound **1** was not stable, and reverted to 2-aldrithiol, thiolpyridine and aminoethanethiol to some extent. The solvents were removed by rotavap, and 100 mg of oily, yellow liquid remained. Some precipitate (but not removed) was formed when the oily liquid was dissolved in chloroform. One gram 1,1'-carbonyldiimidazole was added and at the same time, the precipitate disappeared. Compound **2** was isolated by preparative TLC (silica gel G) and

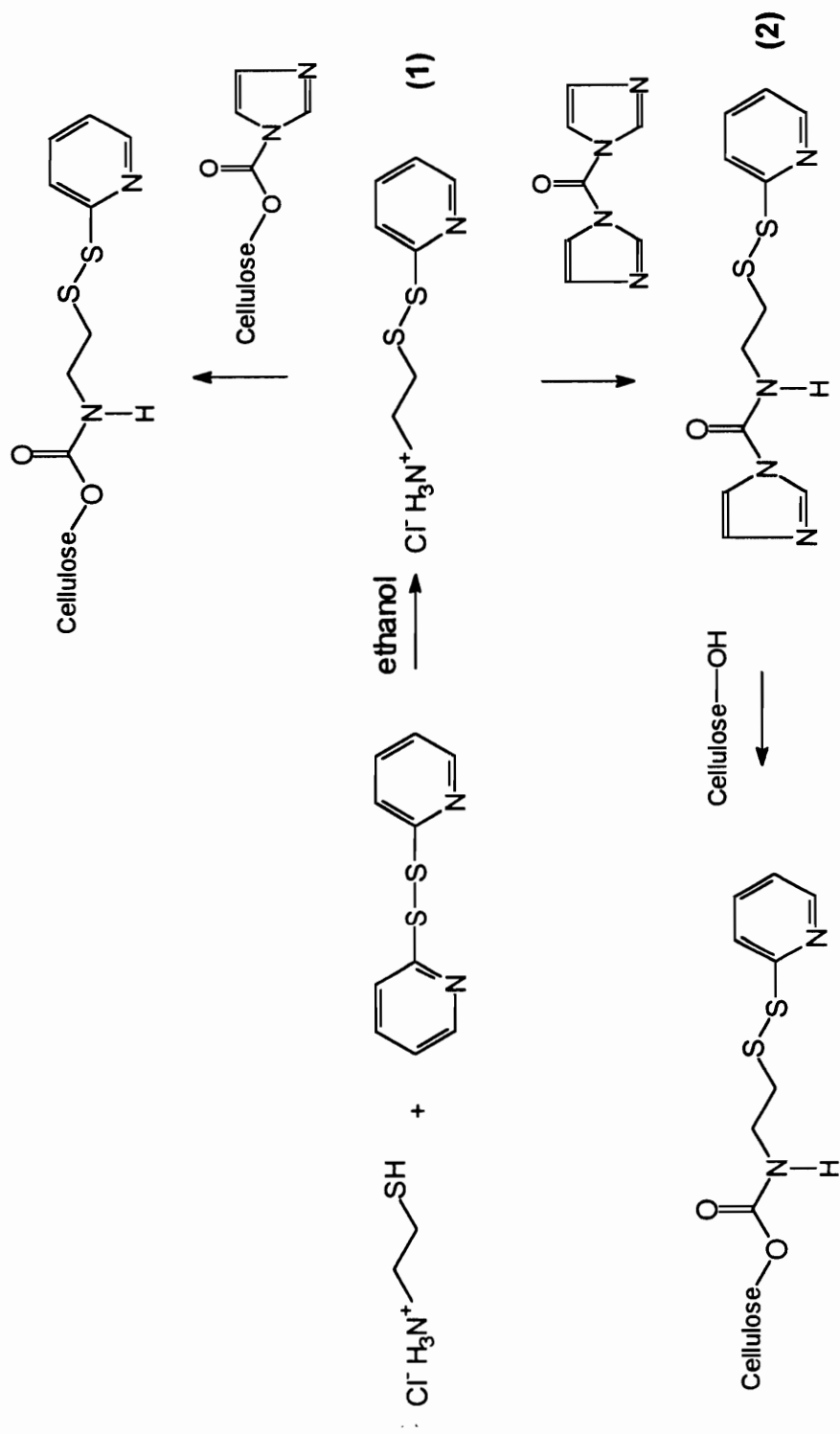


Figure 6. 1,1'-carbonyldiimidazole activation, S protection pathway.



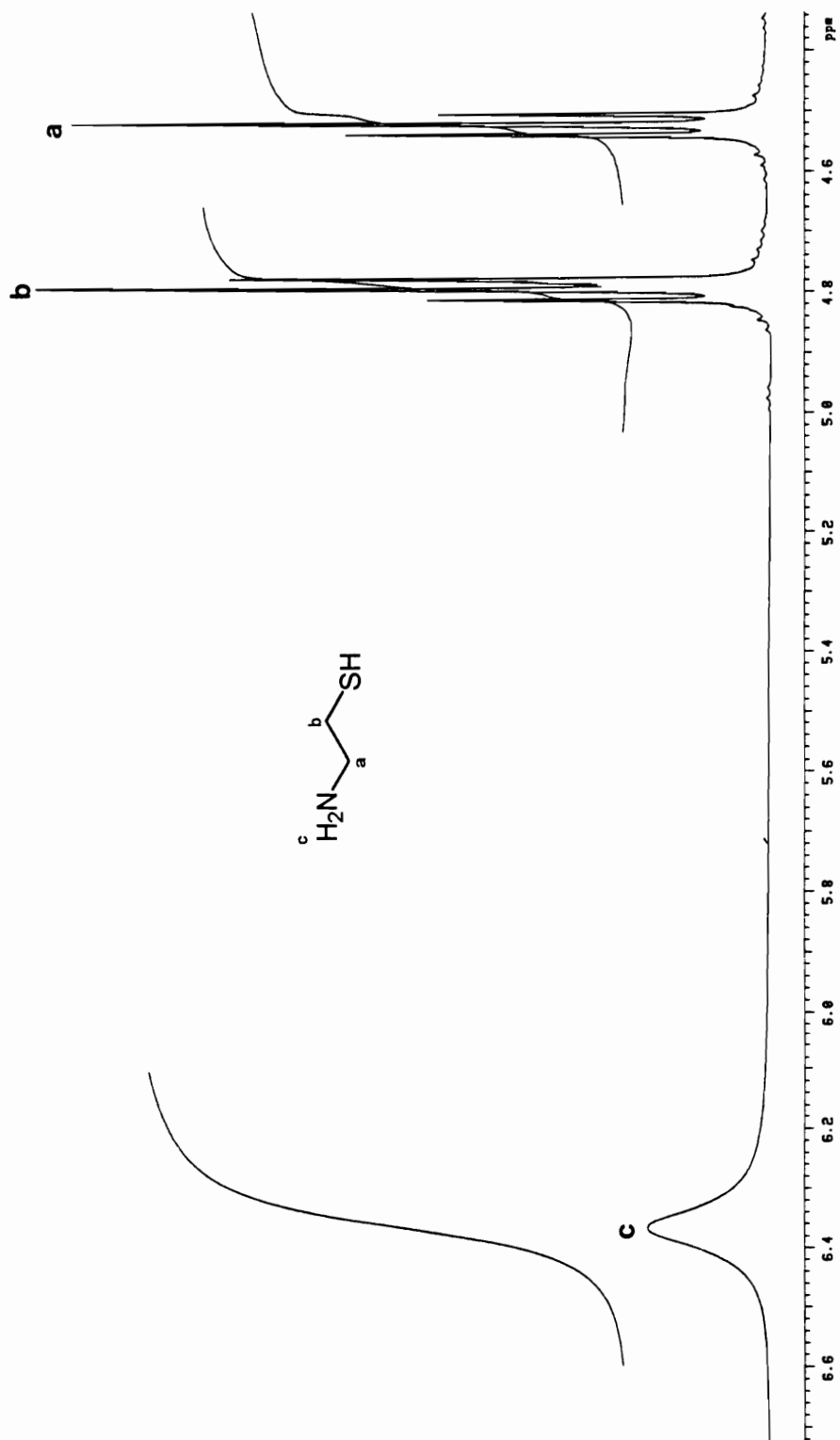


Figure 7.  $^1\text{H-NMR}$  spectrum of aminoethanethiol.

identified by NMR (Figure 8). The same reaction was also carried out in EtOH/Acetic acid 98:2 (v/v), using acetic acid as component of the eluent for separation of compound **3** on silica gel G (Figure 9). Adding 1,1'-carbonyldiimidazole resulted in an acetylation reaction leading to compound **4** (Figure 10).

*2-oxothiazolidine protection of -SH* (Figure 11). One gram of aminoethanethiol was dissolved in 10 mL pyridine and 7 mL THF. 1,1'-carbonyldiimidazole (1575 mg) was slowly added to the pyridine/THF with continuous stirring. The reaction mixture was refluxed for 16 h. The solvent was evaporated in a rotavap, and the residue was redissolved in pyridine/THF. The solution was then passed through an amberlite column, and the effluent evaporated until dry in a rotavap. The residue consisted of a yellow, oily liquid, and a transparent precipitate. Thirty milligrams of the yellow oily liquid was dissolved in 1 mL chloroform:methanol (95:5, v/v). The solution was spotted on a preparative silica gel G plate, and developed using chloroform:methanol (95:5, v/v). A band ( $R_f = 0.3$ ), was scraped of the plate and extracted with chloroform. A white crystalline powder remained following evaporation of the chloroform. The product was identified as 2-oxothiazolidine by H-NMR and  $^{13}\text{C}$ -NMR (Figure 12). 4-phenyl-2-butanol was added to 2-oxothiazolidine at 60°C. The same reaction was also carried out with sodium methoxide instead of 4-phenyl-2-butanol.

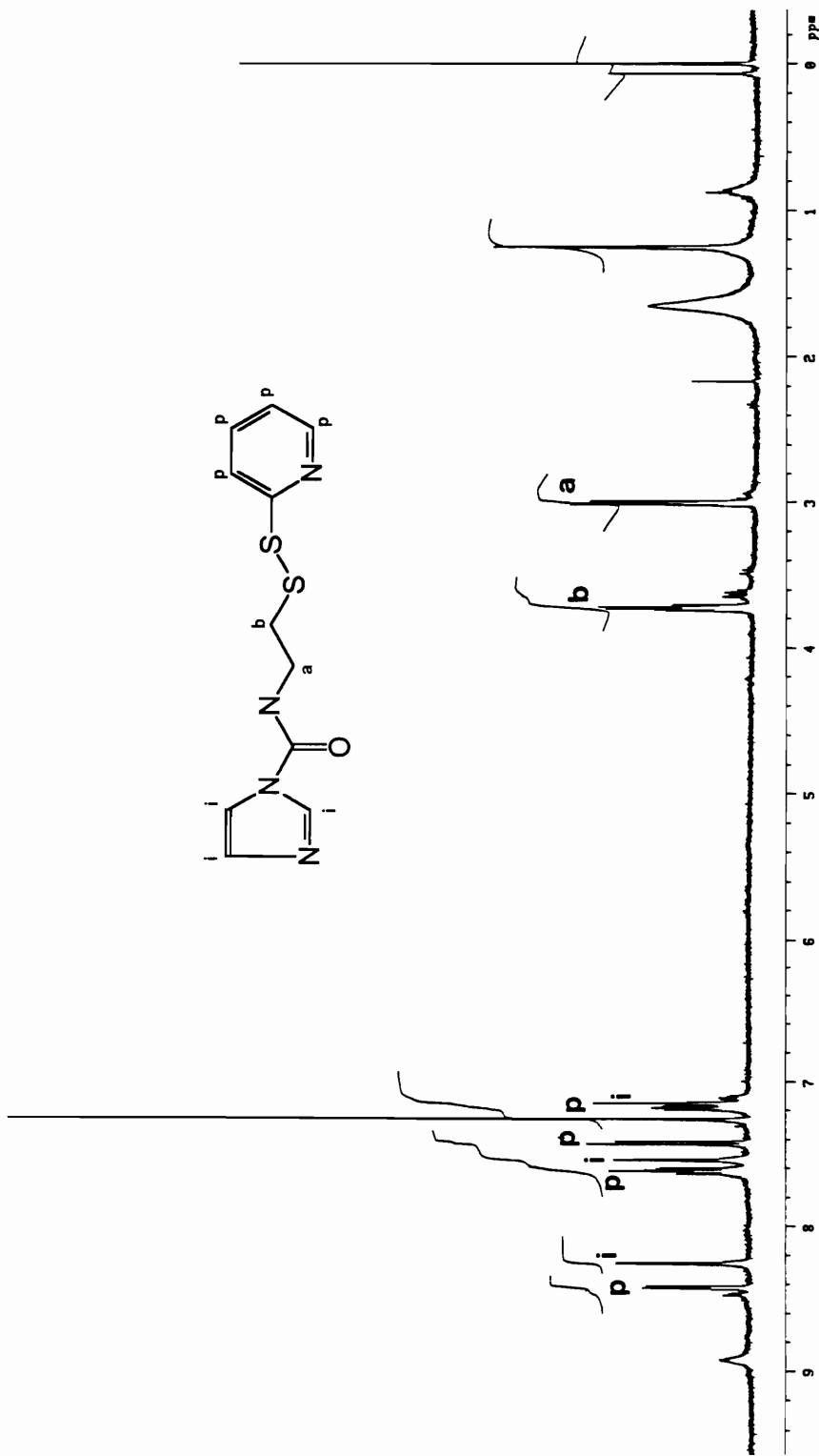


Figure 8. <sup>1</sup>H-NMR spectrum of compound 2.

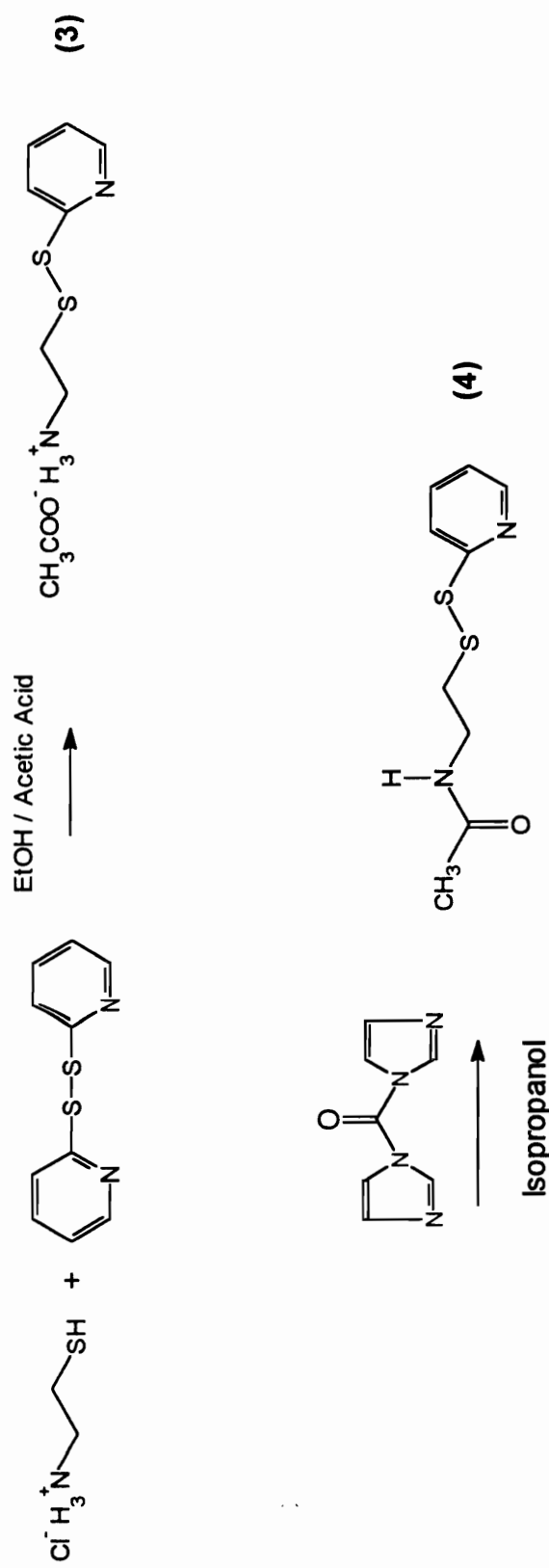


Figure 9. Acetylation reaction.

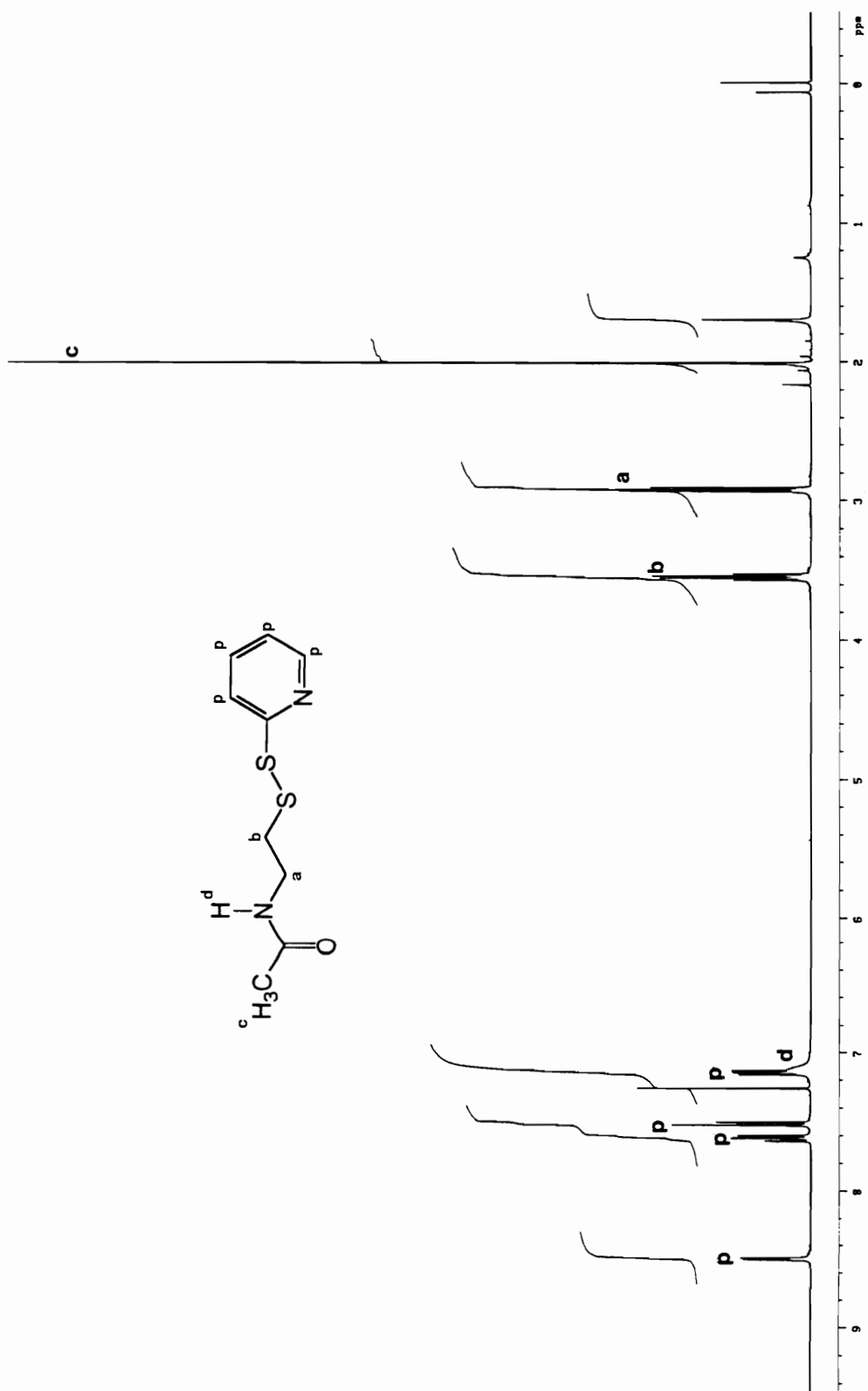


Figure 10. H-NMR spectrum of compound 4.

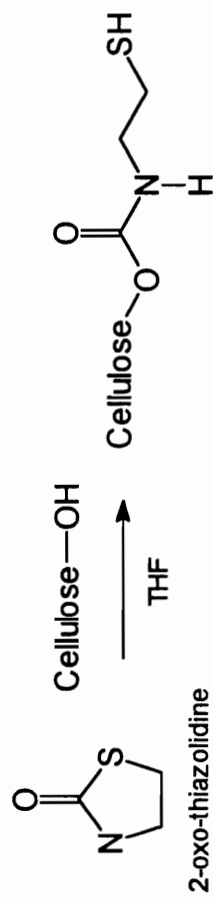
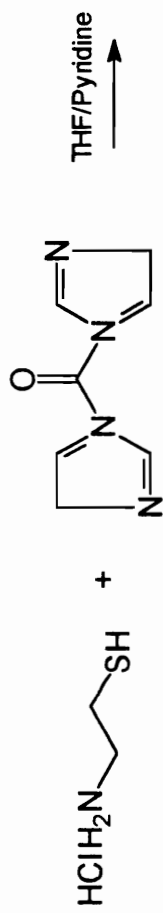


Figure 11. 2-Oxothiazolidine pathway

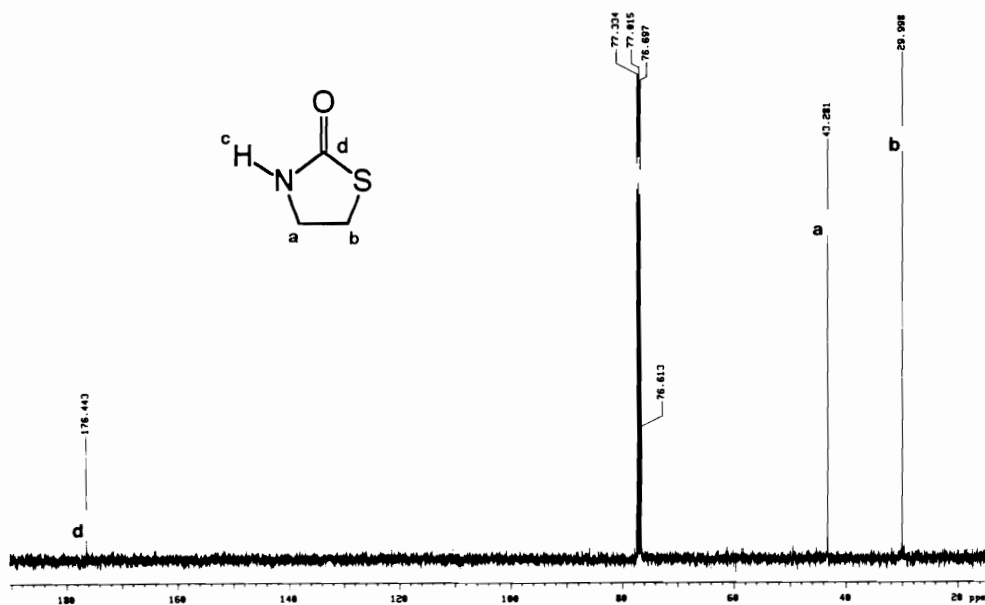
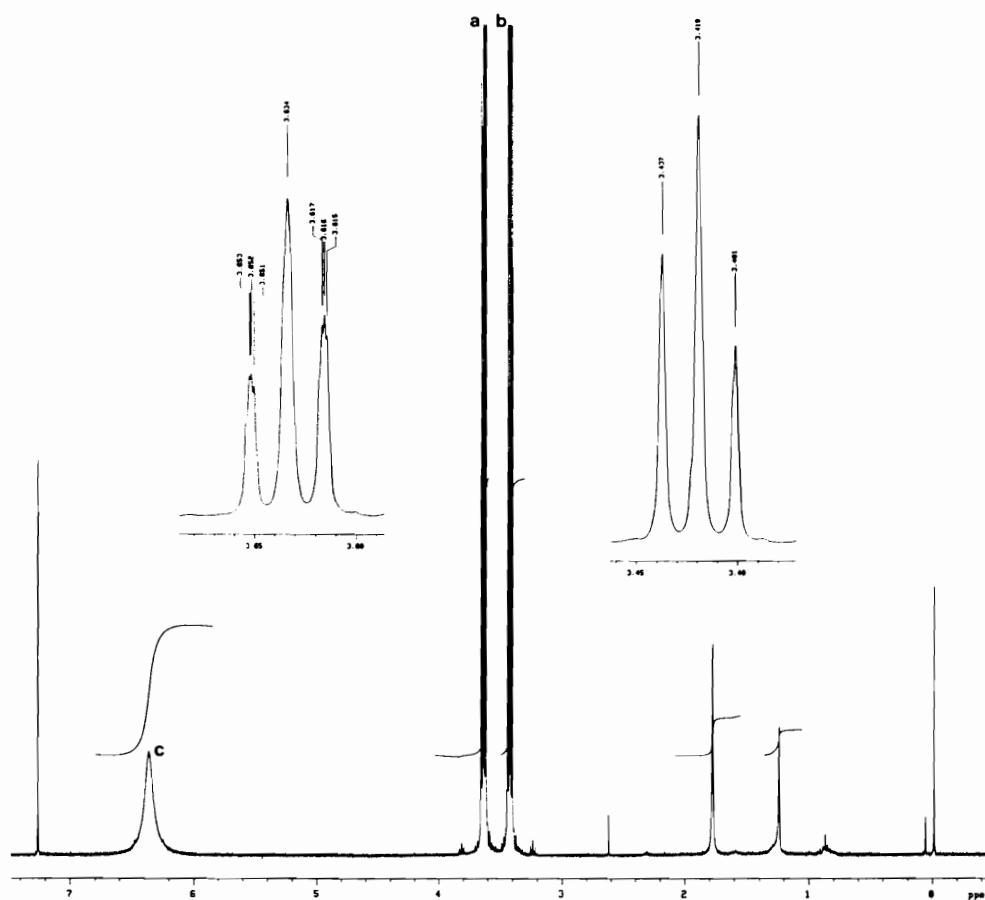


Figure 12. H-NMR spectrum (top) and  $^{13}\text{C}$ -NMR spectrum of 2-oxothiazolidine.

*Carboxymethylation pathway* (Figure 13). Forty milliliters of cellulose beads were washed with 100 mL 0.1M NaCl and suspended in 50 mL 0.1M NaCl in a 125 mL Erlenmeyer flask. Fifty mL of 6.0M NaOH was added, together with 11.648 g sodium chloroacetate. The mixture was placed on an incubation shaker at 25 °C. After one hour, the beads were thoroughly washed with 0.1M NaCl. The beads were split into 4 portions of 10 mL each. Each portion was placed in a 125 mL Erlenmeyer flask and suspended in 25 mL deionized water. The flasks were placed on an incubation shaker at 25 °C and 1.42 g of aminoethanethiol was added. When all aminoethanethiol was dissolved, 2.4 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), a water soluble coupling agent, was slowly added. The mixtures reacted for 2, 4, 8, and 16 h. The derivatized beads were washed with water (pH 10) and then rinsed with distilled water.

*Bis-oxirane pathway*. (Figure 14). Twenty-five milliliters of 1M NaOH was added to each of four 125 mL Erlenmeyer flasks containing 10 mL each of cellulose beads. Each flask received 5 mL of 1,4 butanediol diglycidyl ether. The flasks were placed on an incubation shaker (25 °C) and incubated for 2, 4, 8, or 18 h. At the end of the incubation period, the beads were thoroughly washed with 0.1M NaHCO<sub>3</sub> and then resuspended in 70 mL of 0.1M NaHCO<sub>3</sub>. Hydrogen sulfide, produced through the reaction of ferrous sulfide with 12N H<sub>2</sub>SO<sub>4</sub>, was dispersed through the bead suspension for 2 h. The beads were



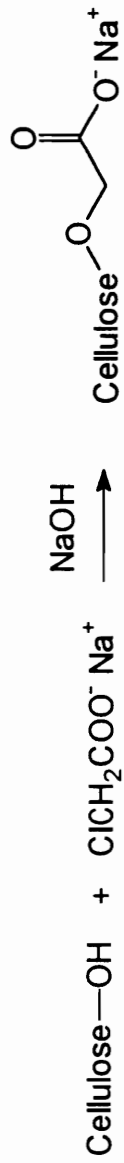


Figure 13. Carboxymethylation pathway



then washed with water and the amount of SH groups was determined as described below.

*Carbonyldiimidazole pathway* (Figure 15). Carbonyldiimidazole (7.4 g) was added to THF dried cellulose beads (40 mL) suspended in 25 mL THF. After 2 h, on a gently swirling incubation shaker (25 °C), the beads were washed with DMF and split into four 10 mL portions. The beads were suspended in 10 mL DMF and 1.7 g aminoethanethiol was added. Reaction times for the beads were 2, 4, 8, and 16 h at 25 °C. Following the reaction, the beads were first washed with 200 mL of pH 4 water and then with 200 mL of water (pH 10). The beads were allowed to stand in pH 10 water overnight at 25 °C. Finally the beads were washed with deionized water, and the sulfhydryl content was determined as described below.

*Quantification of sulfhydryl groups.* One milliliter of derivatized beads was transferred to a 10mL test tube for -SH content determination. The beads were washed 5 times with 5 mL of deionized water and finally resuspended in 5 mL of water. Sodium borohydride (50 mg) was added to the beads to reduce all disulfide bonds. After one hour, the beads were 5 times washed with 5 mL water and 5 times with 5 mL of methanol and resuspended in 5 mL of methanol. 2-aldrithiol (245 mg) was then added to the suspended cellulose beads. The tubes were gently shaken and allowed to stand overnight (16 h).

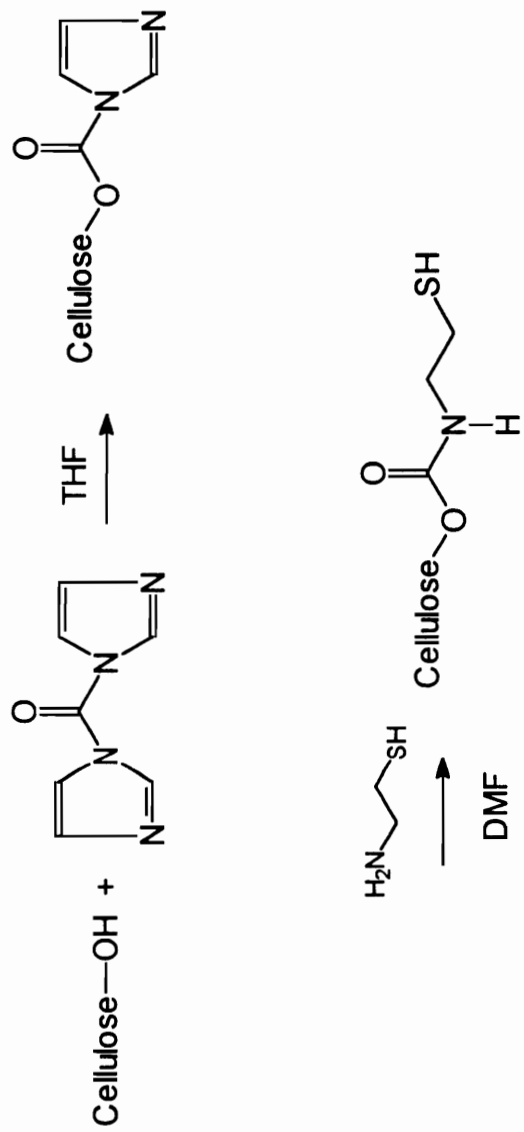


Figure 15. 1,1'-carbonyldiimidazole pathway.

The beads were again washed with 5 mL methanol (5 x) and 5 mL water (5 x). At this point, all the free SH groups should be protected with the UV active thiolpyridine group. Five mL of water and 170 mg of dithiothreitol, a reducing agent, was added to the cellulose beads to release the thiolpyridine (Figure 16).

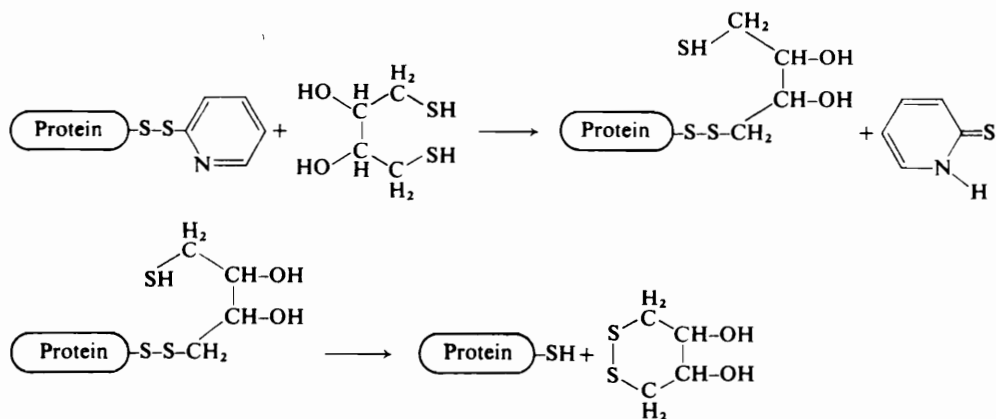


Figure 16. Deprotection of thiolpyridine protected sulfur using dithiothreitol (Carlsson et al. 1978).

Following one hour incubation, the supernatant was collected and placed in a 50 mL volumetric flask. Five additional washings with 5 mL of water were performed. All the washes were combined in the same 50 mL volumetric flask. The volumetric flask was brought to volume, and absorbancy at 343 nm was measured using a Beckman spectrophotometer (Model DU-7). The amount of released thiolpyridine was quantified using the external standard method. The amount of SH is equivalent to the amount of thiolpyridine released.

## RESULTS AND DISCUSSION

### **Reaction of metolachlor with mercaptopropionic and aminobutyric acid.**

The results clearly show that metolachlor did not react with aminobutyric acid at all temperature and pH combinations (Figure 17). Reaction between metolachlor and mercaptopropionic acid was dependent on pH and temperature (Figure 18) and followed pseudo first order reaction kinetics ( $r^2$  between 0.78 and 0.99). The reaction rate was greatly enhanced by increasing the temperature and the pH (Figure 19). Increased reactivity at higher pH is most likely the result of an increase in nucleophilic character of the sulfhydryl group. The metolachlor-mercaptopropionic conjugate was isolated on preparative silica gel G plates using hexane:ethylacetate:methylene chloride (6:3:1, v/v/v) as mobile phase. NMR spectra of metolachlor and the conjugate are presented in Figure 20 and 21, respectively. Based on these results, I concluded that the most effective nucleophile for conjugate formation with metolachlor was the -SH group.

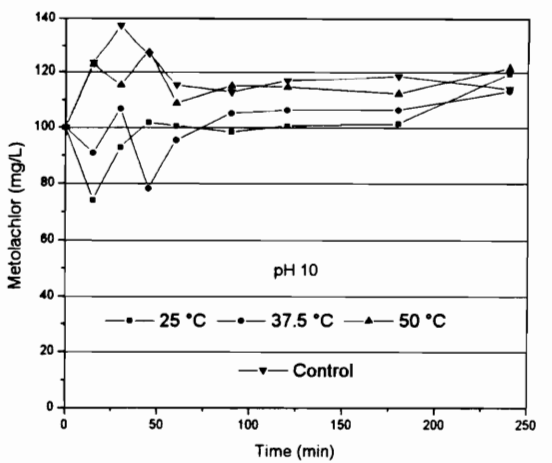
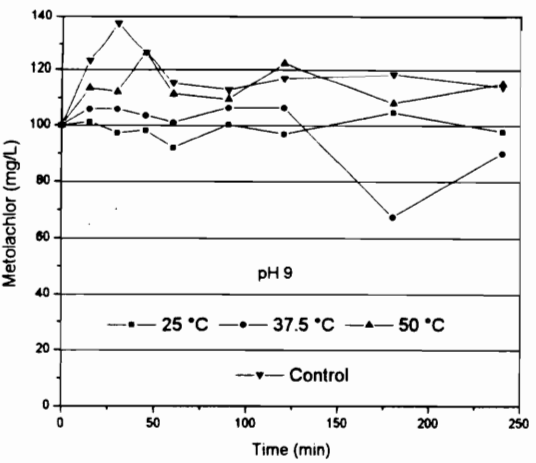
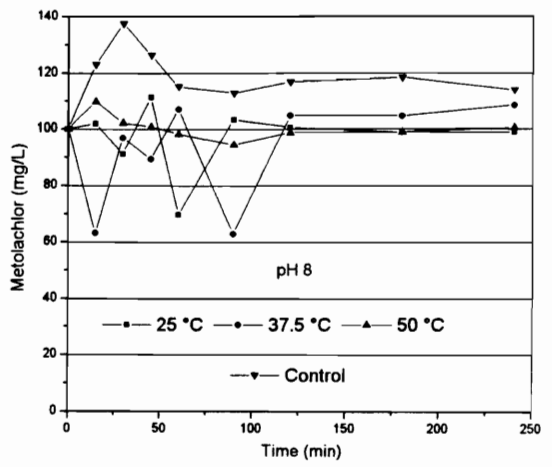
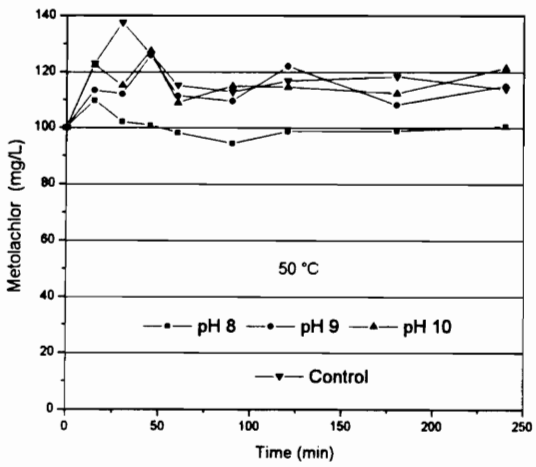
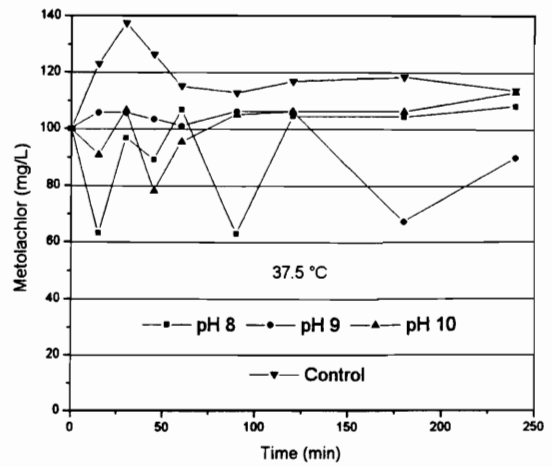
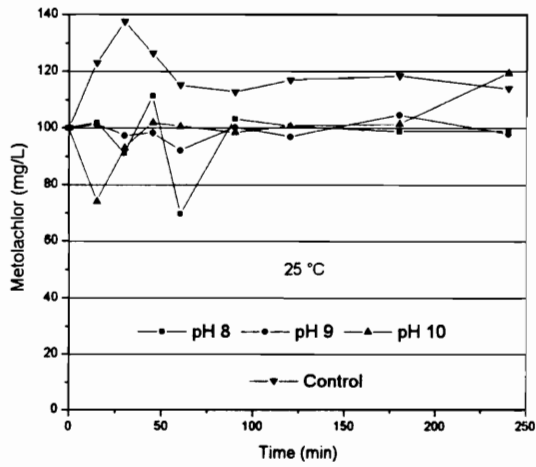


Figure 17. Reaction of metolachlor and aminobutyric acid at different temperatures and pH.

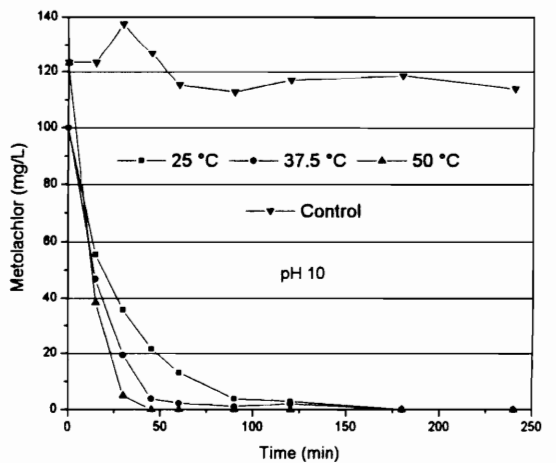
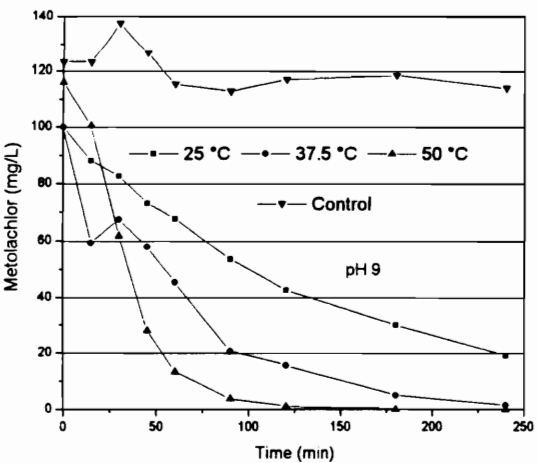
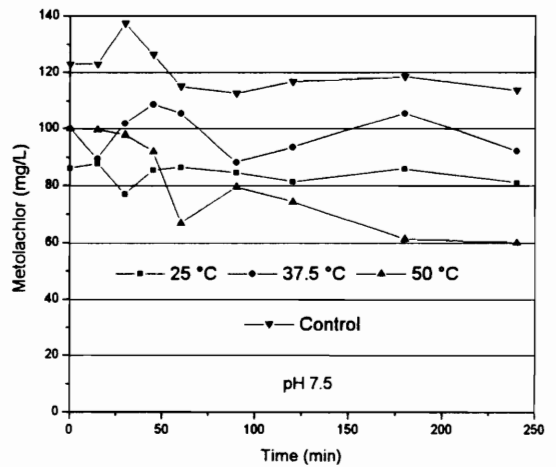
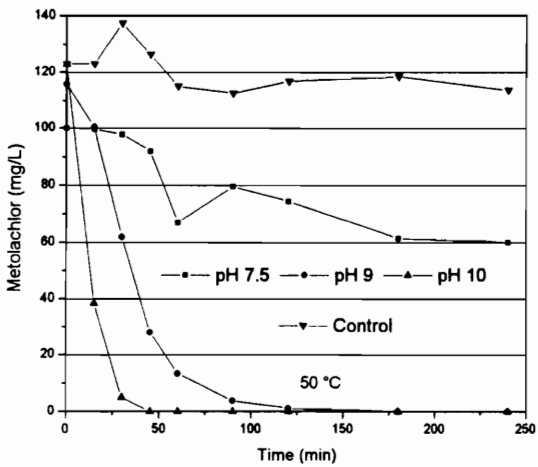
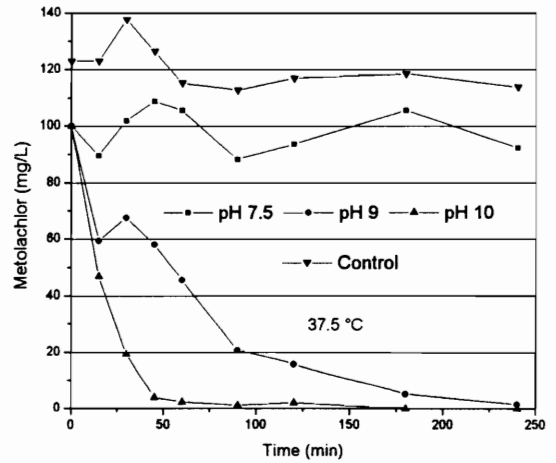
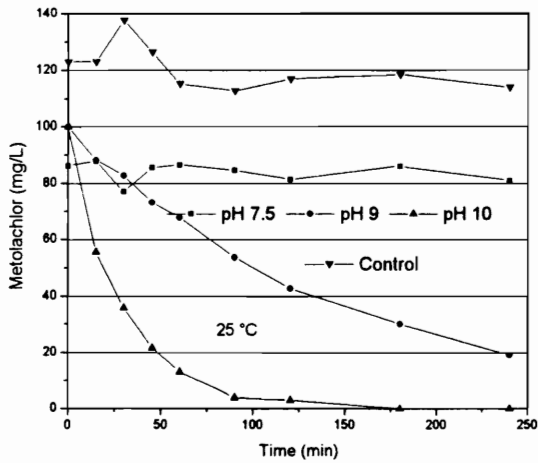


Figure 18. Reaction of metolachlor and mercaptopropionic acid at different temperatures and pH.



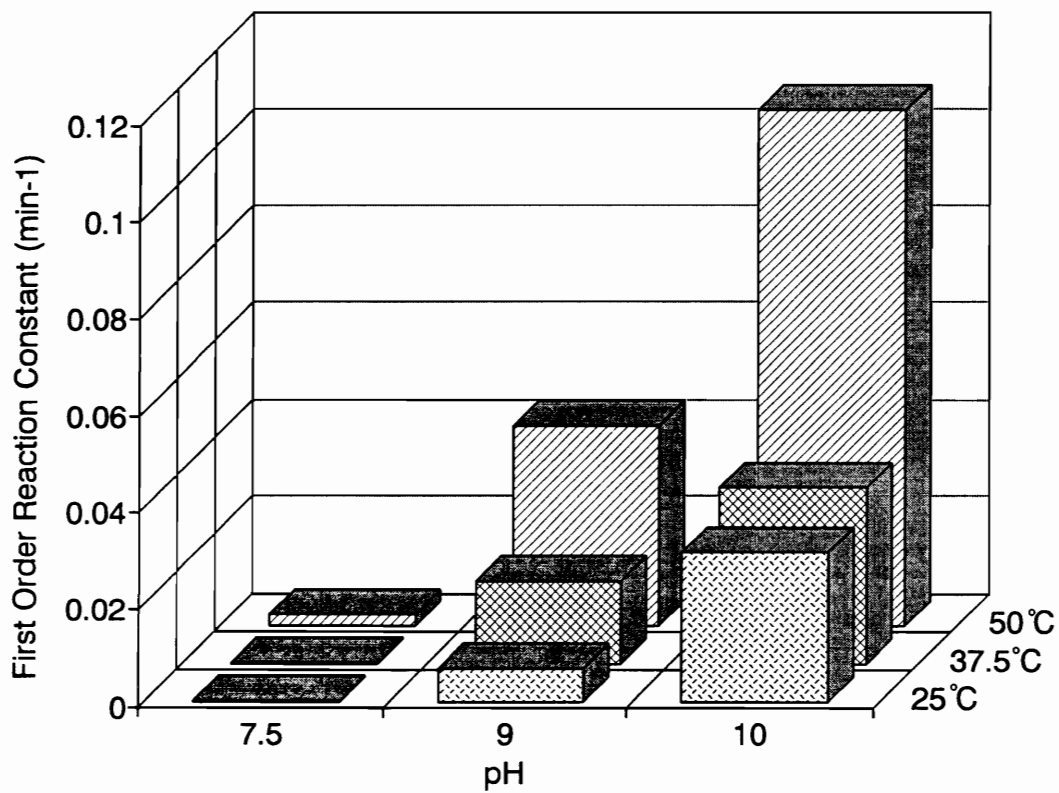


Figure 19. Reaction rate constants for the reaction of metolachlor with mercaptopropionic acid at different temperatures and pH.

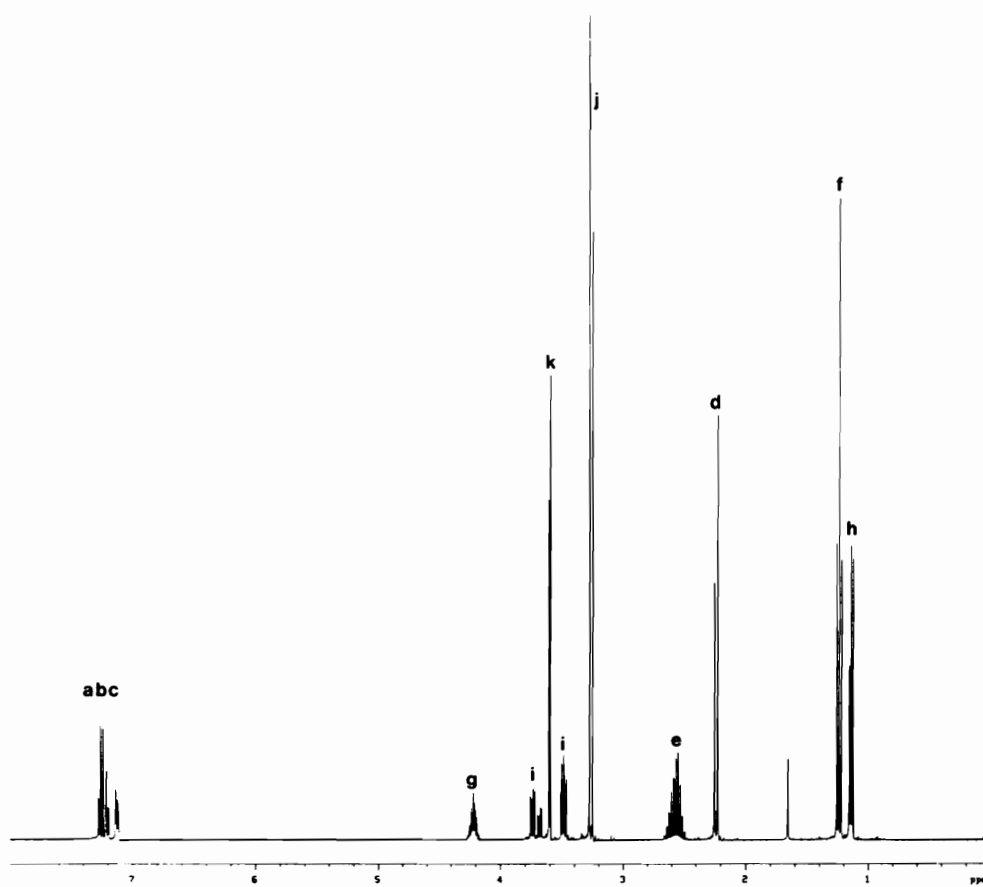
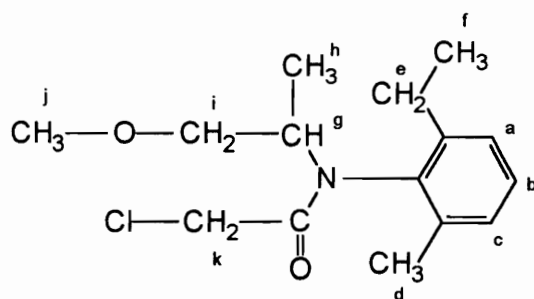


Figure 20. H-NMR spectrum of metolachlor.

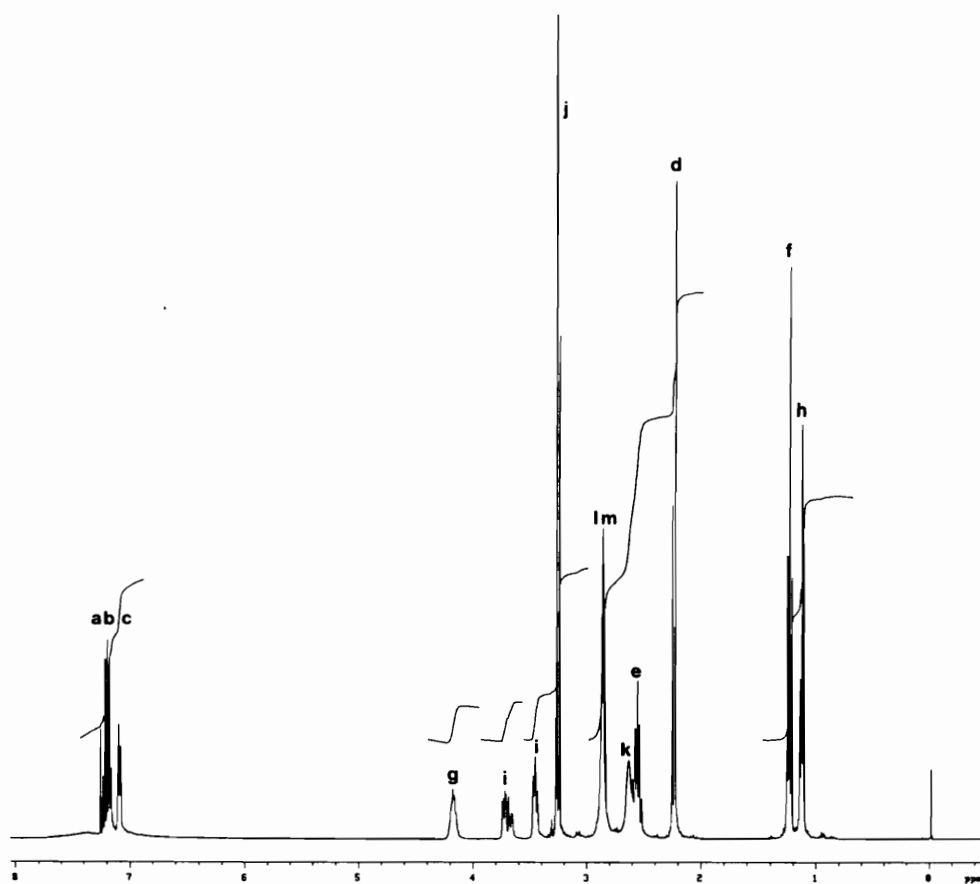
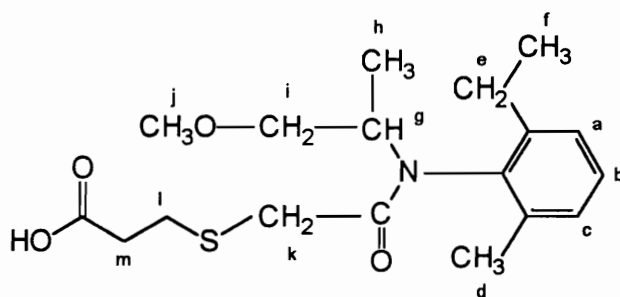


Figure 21. H-NMR spectrum of metolachlor conjugate.

## **Derivatization of cellulose beads.**

I did not attempt to derivatize any beads using compound **1** or **2** (carbonyldiimidazole + SH protection pathway). This was primarily because I was unable to purify compound **1**. The large amounts of remaining thiolpyridine, from the SH protection step, would interfere with further reactions, probably leading to numerous side products, thus reducing product yields.

No SH groups were introduced on the cellulose beads by the oxothiazolidine pathway. The idea was to react 2-oxothiazolidine with free hydroxyls on the cellulose beads. I assumed that 2-oxothiazolidine would selectively open between the carboxyl group and the sulfur, resulting in a free sulfhydryl group. However, I was not able to open 2-oxothiazolidine with 4-phenyl-2-butanol and sodium methoxide even at elevated temperatures (60°C). Therefore I decided not to attempt derivatizing cellulose beads using 2-oxothiazolidine.

The amounts of SH functionality obtained by the carboxymethylation, bis-oxirane, and carbonyldiimidazole pathways are shown in Figure 22. The carboxymethylation pathway resulted in a maximum yield of 1  $\mu$ moles SH/mL beads (8 h reaction between the carboxymethylated cellulose beads and aminoethanethiol). No more than 8  $\mu$ moles SH/mL beads were introduced by the bis-oxirane pathway (4 h reaction between cellulose beads and 1,4

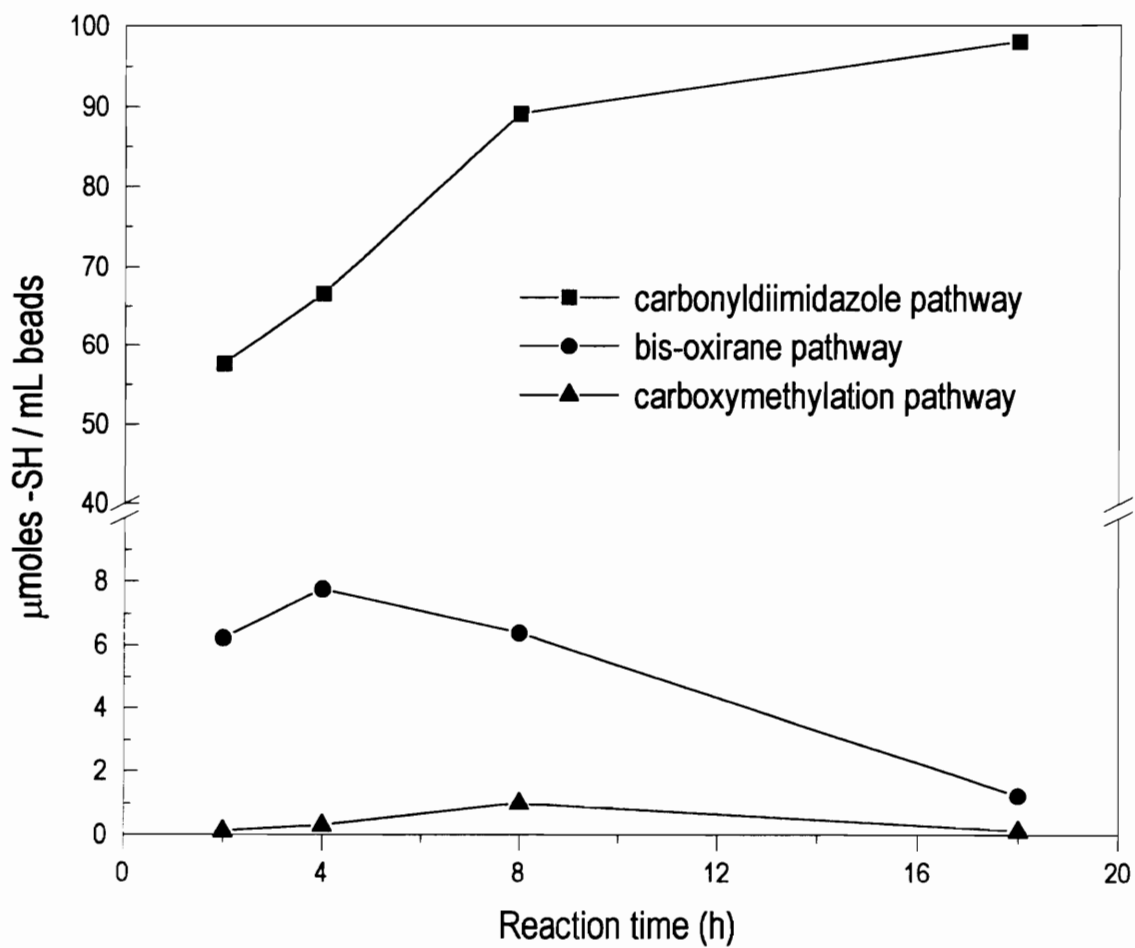


Figure 22. Introduction of -SH groups onto cellulose beads.

butanediol diglycidyl ether). The amount of -SH groups decreased with longer reaction times.

The most successful derivatization method was the carbonyldiimidazole pathway without prior protection of the sulfhydryl group on aminoethanethiol. A yield of approximately 100  $\mu$ moles SH/mL beads was obtained (reaction time of 18 h). The amount of -SH introduced onto the beads increased with reaction time. During the reaction of the carbonyldiimidazole activated cellulose beads with aminoethanethiol, thiocarbamate linkages may have been formed, resulting in free  $\text{NH}_2$  groups instead of SH groups. The  $\text{NH}_2$  groups were removed through hydrolysis of the thiocarbamate bonds under basic conditions (pH 10). An essential element for the successful activation of the cellulose beads using 1,1'-carbonyldiimidazole are anhydrous reaction conditions. Thus, all traces of water were removed from the cellulose beads (THF dried beads). If water were present, it would have reacted with 1,1'-carbonyldiimidazole, reducing the efficiency of the activation step. The SH cellulose beads produced by this method will be referred to as ethanethiol-cellulose beads.

## CONCLUSIONS

The results clearly indicate that the sulfhydryl group is a good nucleophilic agent to react with metolachlor. The reactivity is greatly enhanced

with increasing pH and temperature. Of the five methods evaluated to introduce a sulfhydryl group on beaded cellulose, only two were effective. Under the given conditions, the bis-oxirane method resulted in a sulfhydryl content of 8  $\mu$ moles SH per mL beads. The most effective method was the carbonyldiimidazole pathway, leading to a sulfhydryl content of 100  $\mu$ moles per mL beads. This method was used to produce large quantities of ethanethiol-cellulose beads. The derivatized cellulose beads will be tested for metolachlor adsorption-immobilization in batch and column operations.

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## **CHAPTER IV**

### **Removal of the Herbicide Metolachlor from Water Using Ethanethiol-cellulose Beads**

## ABSTRACT

The use of ethanethiol-cellulose beads to adsorb metolachlor from aqueous solutions was investigated in batch and fixed-bed column studies. Ethanethiol-cellulose beads, with an SH content of 23-160  $\mu$ moles SH per mL bedvolume, were synthesized by a novel method using 1,1'-carbonyldiimidazole as an activating agent followed by reaction with aminoethanethiol. Batch sorption studies revealed that ethanethiol-cellulose beads had adsorptive capacities ranging between 4 and 58 mg metolachlor per mL bedvolume, depending on the pH and temperature. The higher adsorptive capacities were obtained at elevated temperature and pH. Ethanethiol-cellulose beads (at elevated temperature and pH) had similar adsorptive capacities for metolachlor as Ambersorb adsorbent 572 (Rohm and Haas Chem. Co.). Sorption of metolachlor onto ethanethiol-cellulose beads was most likely the result of chemisorption. Even though results from the fixed-bed column studies revealed that the derivatized beads possessed a large capacity for metolachlor, high effluent quality could not be maintained over extended column operation. Not surprisingly, initial effluent quality decreased with increasing flow rates. Unmodified cellulose beads showed no adsorptive capacity for metolachlor in either batch sorption studies or fixed-bed column studies. Our results showed that ethanethiol-cellulose beads can potentially be used in cleanup procedures

for water containing metolachlor, and possibly other electrophilic pesticides.

## INTRODUCTION

Polysaccharide beads are widely used as support material in affinity chromatography. The presence of free hydroxyl groups render the beads susceptible to numerous derivatization procedures. Extensive literature reviews on the chemical modification of cellulose and beaded carbohydrates (e.g., agarose and cellulose) are available (Peng et al., 1987; Boeden et al., 1991; and Gemeiner et al., 1989). Derivatized beaded polysaccharides are normally used as a tool for purification and immobilization of biomolecules (e.g., DNA, sugars, proteins, and enzymes).

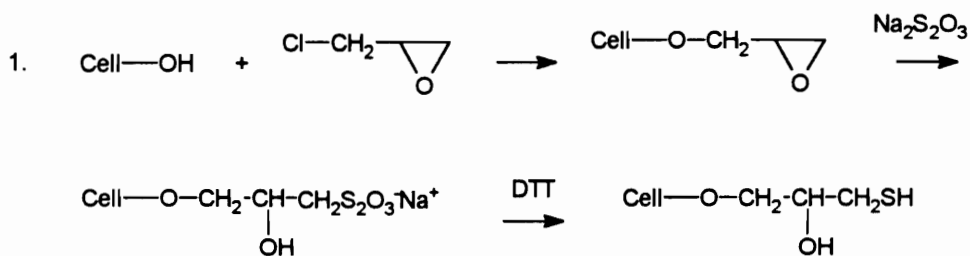
Not much research has been conducted on the use of derivatized cellulose beads as an adsorbent for wastewater cleanup. Thiol derivatized cellulose beads have been used for the removal of Cu, Cd, and Hg from milk (Roh et al., 1975; 1976). They reported that up to 85% of Hg could be removed from milk. Marchant (1974) evaluated the use of thiol derivatized cellulose beads for Hg adsorption from aqueous solutions. Sixty bedvolumes of aqueous Hg solution of 1 ppm could be treated at 50 mL/h before breakthrough was reached (5 ppb). Beaded cellulose sulfonic acid cation exchangers have been successfully used for the production of ultra-pure water (Matejka and Eliasek,

1984; and Matejka et al., 1984).

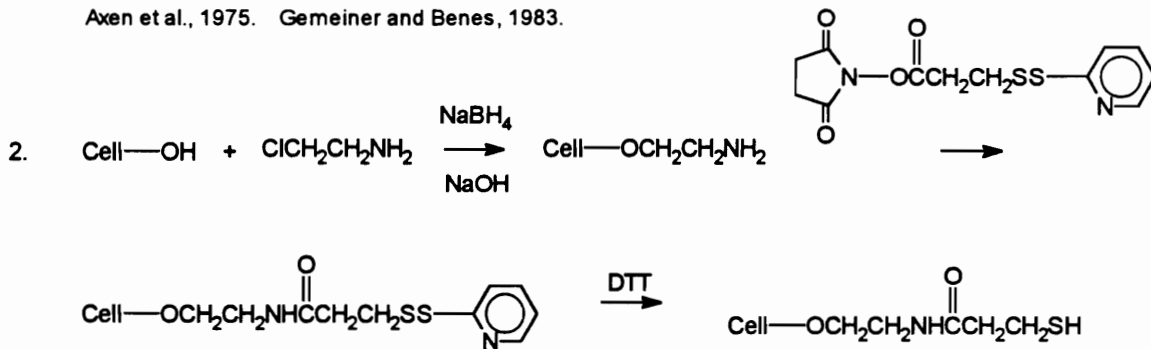
To the authors' knowledge, the use of derivatized cellulose beads for the removal of pesticides from contaminated water has not been investigated. Derivatized cellulose beads may prove useful adsorbents, provided that (i) inexpensive methods to derivatize cellulose beads can be developed, (ii) the derivatized beads possess a high adsorptive capacity for the contaminants, and (iii) environmentally safe and inexpensive disposal methods for the spent beads are available. Adsorptive capacity and selectivity is primarily determined by the extent and type of derivatization. Beaded cellulose containing nucleophilic groups (e.g., thiols) could prove a successful chemisorbent to decontaminate water containing electrophilic organic contaminants.

Some of the most heavily used herbicides in the United States, atrazine, simazine, alachlor, and metolachlor (Gianessi and Puffer, 1991), all contain electrophilic moieties (e.g., the  $\alpha$  carbon of the chloroacetamide group in alachlor and metolachlor) and thus could be immobilized (chemisorbed) on a thiol containing support. These four compounds are not only some of the most commonly used herbicides, they are also the most frequently cited as water pollutants (Glotfelty et al., 1984, and Pereira et al., 1992).

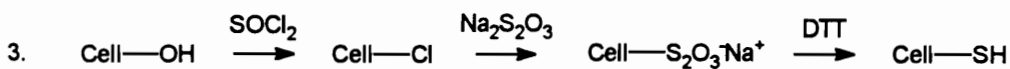
Several procedures have been described to introduce thiol groups onto cellulose (Fig. 1). I synthesized ethanethiol-cellulose beads using a novel method that is superior to the previous methods because of its relative



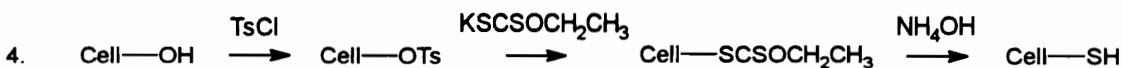
Axen et al., 1975. Gemeiner and Benes, 1983.



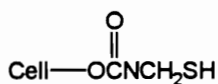
Oscarsson et al., 1992.



Gemeiner and Zemek, 1981.

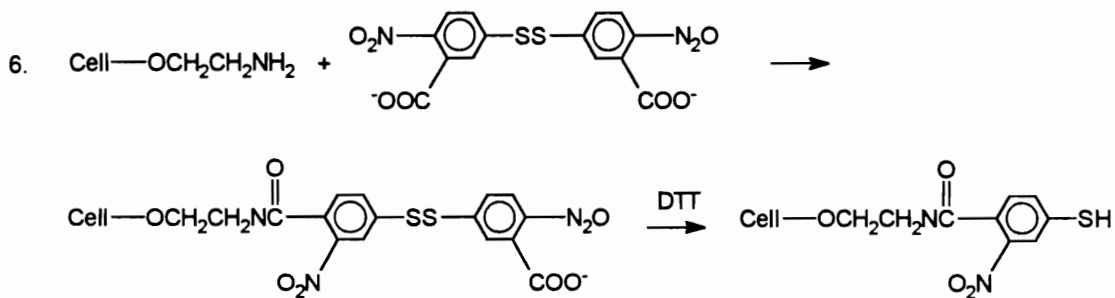


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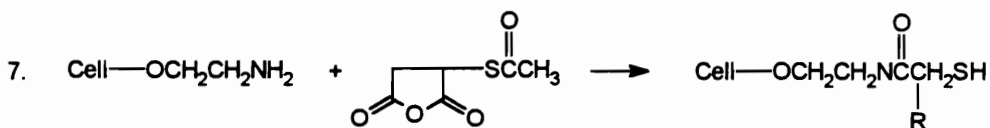


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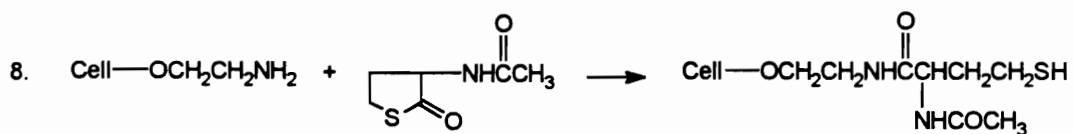
Figure 1. Thiol derivatization of cellulose beads.



Roh et al., 1975.



Roh et al., 1975.



Feist and Danna, 1981.

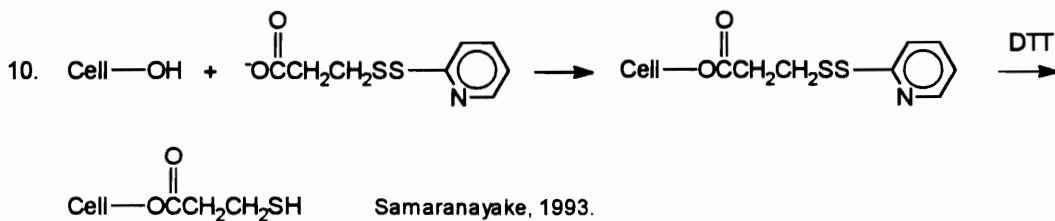
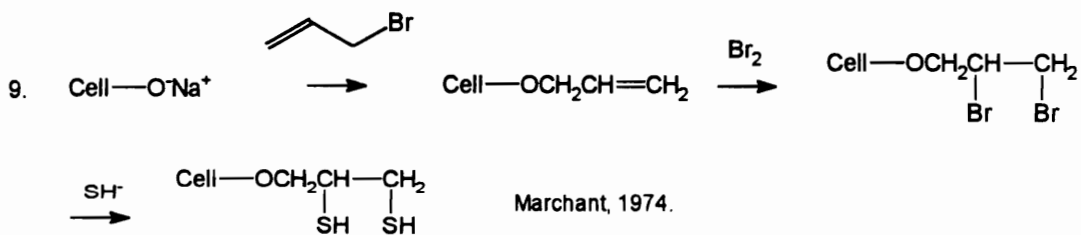


Figure 1. Thiol derivatization of cellulose beads, continued.



simplicity and high yield potential. The method involves an initial 1,1'-carbonyldiimidazole activation step, followed by a reaction with aminoethanethiol. The ethanethiol-cellulose beads were tested for their ability to remove metolachlor from aqueous solution in batch sorption and fixed-bed column studies.

## MATERIALS AND METHODS

**Chemicals.** 2-Aminoethanethiol hydrochloride, 1,1'-carbonyldiimidazole, tetrahydrofuran (THF), *N,N*-Dimethylformamide (DMF), 2-mercaptopyridine, sodiumborohydride, 2,2'-dipyridyl disulfide (2-aldrithiol), and dithiothreitol (DTT) were obtained from Aldrich Chemical Co., Inc. Technical grade (TG) metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] was purchased from Chem Service, West Chester, Penn. Cellulose beads, prepared as described by Kaster et al. (1993), were kindly provided by Dr. W.G. Glasser (Department of Wood Science and Forest Products, Virginia Tech).

**Synthesis of ethanethiol-cellulose beads.** Cellulose beads were dried with THF in a soxhlet apparatus to remove all traces of H<sub>2</sub>O. The THF dried beads were suspended in THF and 9.5 g carbonyldiimidazole was added per 50 mL of beads. The suspension was placed on an incubation shaker at 25 °C for 4 h. After 4 h the beads were placed on a paper filter and rinsed with THF and

then DMF. The DMF washed beads (50 mL) were suspended in DMF and 8.5 g aminoethanethiol-hydrochloride. After 18 h on an incubation shaker at 25 °C, the beads were placed on a paper filter and rinsed with 200 mL of acetic acid-NaOH solution (pH 4.5) and then with 200 mL of carbonate ( $\text{NaHCO}_3$  -  $\text{Na}_2\text{CO}_3$ ) solution of pH 10. The rinsed beads were suspended in pH 10 carbonate solution and placed on an incubation shaker at 25 °C for 18 h. Finally, the beads were rinsed with a 80% ethanol solution and stored in 80% ethanol at 9°C.

**Quantification of -SH groups.** The amount of free thiol groups was determined through initial protection with 2-mercaptopyridine, followed by quantification of the released UV active marker upon reduction with dithiothreitol. One mL of derivatized beads was transferred to a 10mL test tube for -SH content determination. The beads were thoroughly rinsed with deionized water and finally resuspended in 5 mL of water. Sodium borohydride (50 mg) was added to the beads to reduce all disulfide bonds. After one hour the beads were again thoroughly rinsed with water and then methanol before being resuspended in 5 mL of methanol. 2-Aldrithiol (245 mg) was then added to the suspended cellulose beads. The tubes were gently shaken and allowed to stand overnight (16 h). The beads were once again rinsed with methanol and then water. Following this reaction, all free SH groups were protected with the UV active 2-mercaptopyridine group. Dithiothreitol (170 mg), a reducing agent,

was then added to the cellulose beads to release the 2-mercaptopyridine. Following one hour incubation, the supernatant was collected in a 50 mL volumetric flask. Five additional washings with 5 mL of water were performed. All the rinsates were combined in the same 50 mL volumetric flask. The volumetric flask was brought to volume, and absorbancy at 343 nm was measured using a Beckman spectrophotometer (Model DU-7). The amount of released 2-mercaptopyridine was quantified using the external standard method. The amount of SH groups is equivalent to the amount of 2-mercaptopyridine released.

**Sorption isotherms.** Batch-type sorption experiments were conducted to compare the abilities of ethanethiol-cellulose beads (160  $\mu$ moles SH/mL beads) and non derivatized cellulose beads to sorb technical grade (TG) metolachlor. Sorption experiments were conducted at 25 °C and 40 °C, and at three pH levels: 7, 8 and 9. pH 7 buffer was made up of 50 mL 0.1M  $\text{KH}_2\text{PO}_4$  + 29.1 mL 0.1M NaOH. pH 8 and 9 buffer were made up of 50 mL 0.025M tetraborate + 20.5 and 4.6 mL 0.1M HCl, respectively. Initial metolachlor (TG) concentrations were 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, and 450 mg/L. Sorption isotherm studies were initiated by adding 30 mL of metolachlor (TG) solution to 0.2 mL of cellulose or ethanethiol-cellulose beads in a 125 mL Erlenmeyer flask. Only 1 mL of the cellulose or ethanethiol-cellulose beads was used for sorption at pH 7 at 25 °C. The ethanethiol-cellulose beads were treated with

100 mg of NaBH<sub>4</sub> immediately prior to use in order to reduce all disulfide bonds. The Erlenmeyer flasks were placed on an incubation shaker set at 185 rpm for 24 hour. Metolachlor equilibrium concentrations were determined using an HPLC system (LDC Analytical, Riviera Beach, FL.) consisting of a model CM4000 multiple solvent delivery system and a Spectro Monitor 3100 variable wavelength detector. Metolachlor samples were chromatographed on a 25cm Whatman C-18 Partisphere column. The mobile phase consisted of methanol:H<sub>2</sub>O (80:20, v/v) and was delivered at a flow rate of 1 mL min<sup>-1</sup>. Metolachlor was quantified by the external standards method at wave length of 220 nm.

**Fixed-bed column study.** Glass columns (2 cm i.d.) were filled with either 10 mL cellulose (bulk density of 0.023 g/mL) or ethanethiol-cellulose beads (131 μmoles SH/mL beads, bulk density of 0.14 g/mL). Upon packing, the beadvolume was reduced to 7.7 mL The remaining column volume was filled with glass beads. A metolachlor (TG) solution of 200 mg/L, buffered at pH 8, was pumped through the column at flow rates of 5, 10, and 20 bedvolumes per hour (i.e. hydraulic flow rates of 0.204, 0.408, and 0.817 cm min<sup>-1</sup>, respectively). The column and the metolachlor feed solution were held at room temperature (22 ± 1°C) with the exception of one run at 65°C that was performed at a flow rate of 5 BV/h. Effluent was collected in fractions of 2 to 4 mL with a Haake Buchler LC100 automatic fraction collector. Effluent

metolachlor concentrations were determined as described above.

## RESULTS AND DISCUSSION

**Preparation of ethanethiol-cellulose beads.** Four different batches of ethanethiol-cellulose beads, with a thiol content ranging from 23 to 160  $\mu\text{moles SH/mL}$  beads, were produced (Table 1). The lower amount of SH groups introduced in batch 3, and especially batch 4, may have resulted from differences in properties of cellulose beads used and from the presence of  $\text{H}_2\text{O}$ . The cellulose beads used to prepare batch 4 were larger and had a lower solid content (0.097 g/mL versus 0.14 g/mL for batch 1 and 2) thus reducing the total amount of cellulose-OH groups available for thiol derivatization. Addition of carbonyldiimidazole to batch 3 cellulose beads resulted in the formation of  $\text{CO}_2$ , caused by reaction with water, reducing the overall efficiency of the reaction. The THF dried beads used to prepare batch 3 were stored several months prior to derivatization. During this period, THF probably absorbed some water.

A comparison of thiol derivatized cellulose beads, prepared as described above, with other thiol derivatized carbohydrate beads that are commercially available (Sigma Chemical Co., Pharmacia, and ICN), reveals generally superior thiol contents for the carbonyldiimidazole-aminoethanethiol beads (Table 1). Batch 1 beads had 8 times the thiol content compared to the best

**Table 1. SH introduction by different methods.**

Beads	Support	Derivatization procedure	umoles SH per mL beads
Batch 1	Cellulose	carbonyldiimidazole, aminoethanethiol	160
Batch 2	Cellulose	carbonyldiimidazole, aminoethanethiol	141
Batch 3	Cellulose	carbonyldiimidazole, aminoethanethiol	66
Batch 4	Cellulose	carbonyldiimidazole, aminoethanethiol	23
Sigma 1 <sup>a</sup>	Agarose	hydropropyl-2-pyridyl disulfide	20-35
Sigma 2 <sup>a</sup>	Sepharose	cyanogen bromide, glut.-2-pyridyl disulfide	1
Batch a <sup>b</sup>	Cellulose	carboxyethyl-2-pyridyl disulfide	20-32
ICN 1 <sup>c</sup>	Agarose	hydropropyl-2-pyridyl disulfide	25-30
ICN 2 <sup>c</sup>	Agarose	glutathione	25-30
Pharmacia 1 <sup>d</sup>	Sepharose	hydropropyl-2-pyridyl disulfide	20
Pharmacia 2 <sup>d</sup>	Sepharose	glutathione	1

<sup>a</sup> From Sigma Chemical Co.

<sup>b</sup> Synthesized by Dr. G. Samaranyake, Wood Science, VPI.

<sup>c</sup> From ICN biochemicals

<sup>d</sup> From Pharmacia

commercially available beads. Even the batch 4 beads were comparable to most commercially available beads. The carbonyldiimidazole - aminoethanethiol method for thiol derivatization is more convenient than many of the other procedures shown in Figure 1, because (i) the method consists of a convenient two step reaction, (ii) superior to similar thiol contents are obtained, and (iii) a stable carbamate linkage between cellulose beads and thiol containing ligand is formed. A stable attachment of thiol groups is necessary to prevent or minimize leaching of thiol groups (e.g., through hydrolysis) during fixed-bed column operations, especially at elevated pH and temperatures. This is an important property considering the fact that chemisorption of metolachlor by ethanethiol-cellulose beads is favored at higher pH and temperatures. The ethanethiol-cellulose beads could be stored for at least one month in ethanol:water (70:30, v/v) at 9 °C without significant loss of thiol groups.

**Sorption isotherms.** At 40 °C, sorption of metolachlor onto ethanethiol-cellulose beads could be described by the Langmuir isotherm ( $r^2$  between 0.94 and 0.99). At 25 °C, only the isotherm at pH 9 could be adequately described by the Langmuir equation (Figure 2). The isotherms at pH 7 and 8 at 25 °C were less well described by the Langmuir isotherm ( $r^2$  of 0.79 and 0.7, respectively). Increasing temperature and pH increased sorption of metolachlor onto ethanethiol-cellulose beads significantly. This can be explained by increased nucleophilicity of the thiol group at higher pH values. No

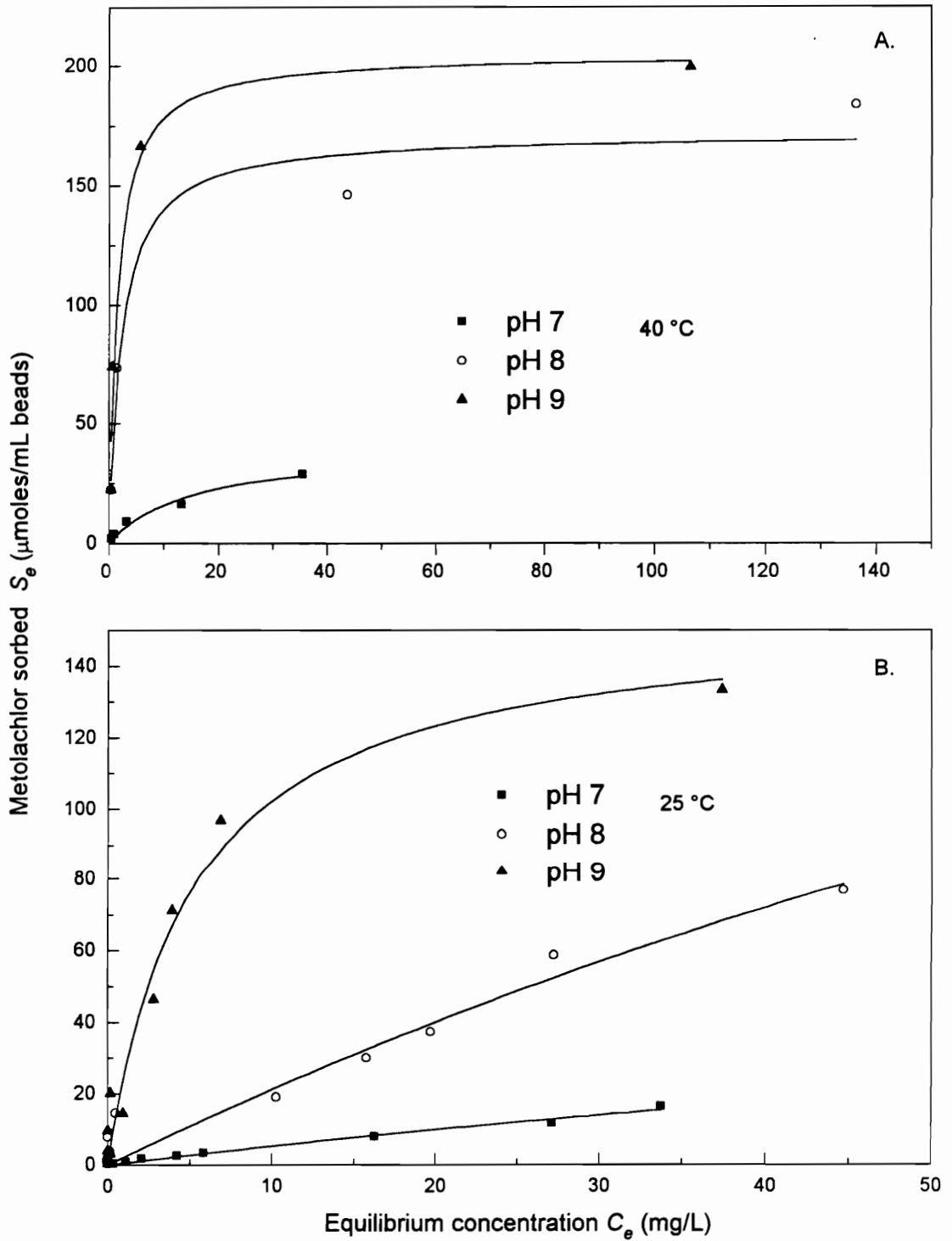


Fig. 2. Adsorption isotherms for adsorption of metolachlor onto ethanethiol-cellulose beads at 40 °C (A) and 25 °C (B).



sorption of metolachlor onto nonderivatized cellulose beads was observed.

Sorption maxima for metolachlor sorption onto ethanethiol-cellulose beads (pH 8 and 9, 40 °C) were similar to the adsorption maxima for adsorption of metolachlor onto Ambersorb adsorbent 572, a carbonaceous, synthetic adsorbent (Table 2). Filtrasorb 400 (activated carbon) performed better than ethanethiol-cellulose beads. The sorption maxima at pH 7 and 8 at 25 °C, and at pH 7 at 40 °C were significantly below the adsorptive capacity that could be expected based on the number of free thiol groups. This was probably the result of an incomplete reaction between metolachlor and thiol groups because of reduced reaction rates at the lower pH and temperatures.

Sorption of metolachlor onto ethanethiol-cellulose beads was primarily the result of chemisorption (Figure 3). If chemisorption would be the only sorption mechanism, then the sorption maximum should be no higher than 160  $\mu\text{moles/mL}$  beads (or  $160 \mu\text{moles/mL} \times .284 \text{ mg}/\mu\text{moles} = 45.44 \text{ mg/mL}$ ). The fact that in some cases slightly higher sorption maxima were observed (Table 2) may indicate the presence of additional sorption mechanisms (e.g., reversible physisorption) or inaccurate quantification of free thiol groups. Two hypothetical sorption mechanisms can be envisioned. The first mechanism involves an initial reversible sorption of metolachlor onto ethanethiol-cellulose beads before chemisorption can occur. The second mechanism involves reversible sorption of metolachlor on top of a chemisorbed layer of metolachlor (Figure 4). In both

**Table 2. Adsorption maxima for metolachlor(TG) adsorption on different adsorbents**

Adsorbent	25 °C			40 °C		
	7	8	9	7	8	9
	pH					
	----- Adsorption maxima (mg/mL BV) -----					
Cellulose	0	0	0	0	0	0
-SH cellulose	6	13	37	10	53	58
Amborsorb 572	52	-	-	-	-	-
Filtrisorb 400	160	-	-	-	-	-

BV = bedvolume

Sorption maximum based on a thiol content of 160 umoles/mL BV = 45.44 mg/mL

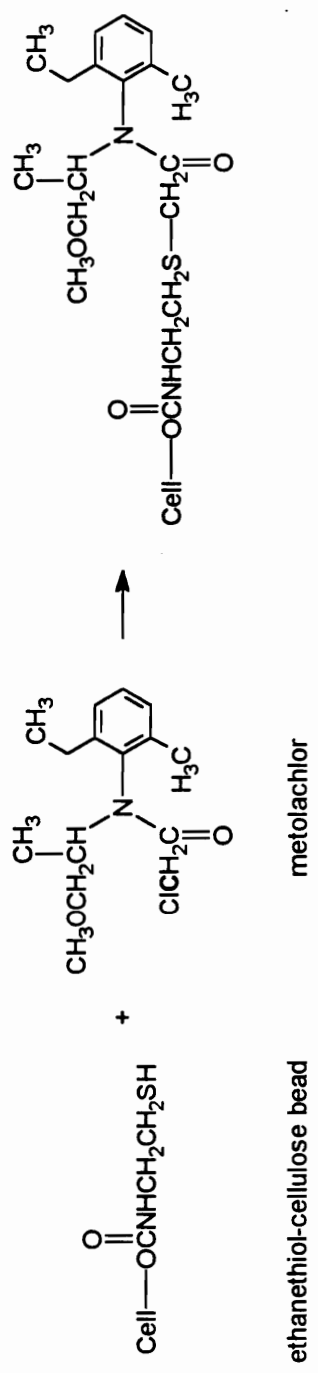


Fig. 3. Chemisorption of metolachlor onto ethanethiol-cellulose beads.

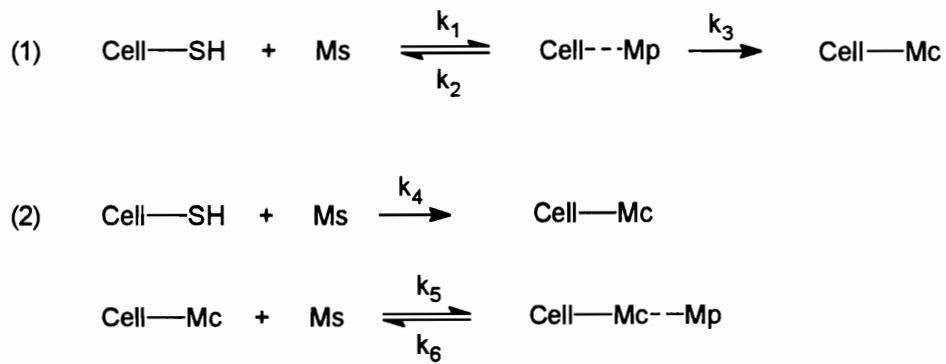


Fig. 4. Hypothetical adsorption mechanisms for metolachlor adsorption onto ethanethiol-cellulose beads. (Ms: metolachlor in solution, Mc: chemisorbed, Mp: physisorbed)

cases, the total amount of metolachlor sorbed consists of a chemisorbed and a physisorbed fraction.

**Fixed-bed column study.** The breakthrough curves for metolachlor sorption onto ethanethiol-cellulose beads and onto nonderivatized cellulose beads are shown in Figure 5. Examination of the curves indicates that (i) no metolachlor was adsorbed onto nonderivatized cellulose beads, (ii) the initial phase of the ethanethiol-cellulose breakthrough curves shifted to the right and was less steep compared with the breakthrough curve for non derivatized cellulose beads, and (iii) the effluent concentration of the ethanethiol-cellulose beads leveled off at a value significantly below the influent concentration.

The shift to the right of the initial phase of the ethanethiol-cellulose breakthrough curves indicates retardation of metolachlor when passing through the column. Also, increasing the flow rate decreases retardation. This behavior is typical for reversible adsorption processes (physisorption).

The fact that effluent concentration for ethanethiol-cellulose beads levels off below the influent concentration, indicates the presence of a "sink" for metolachlor. In this case, the sink is caused by chemisorption of metolachlor. The effluent should reach influent concentration as soon as the "sink" becomes exhausted (i.e., no thiol groups left). The breakthrough curves for ethanethiol-cellulose beads level off at lower effluent concentrations with decreasing flow rates. Increasing the flow rate reduces the residence time of metolachlor in the

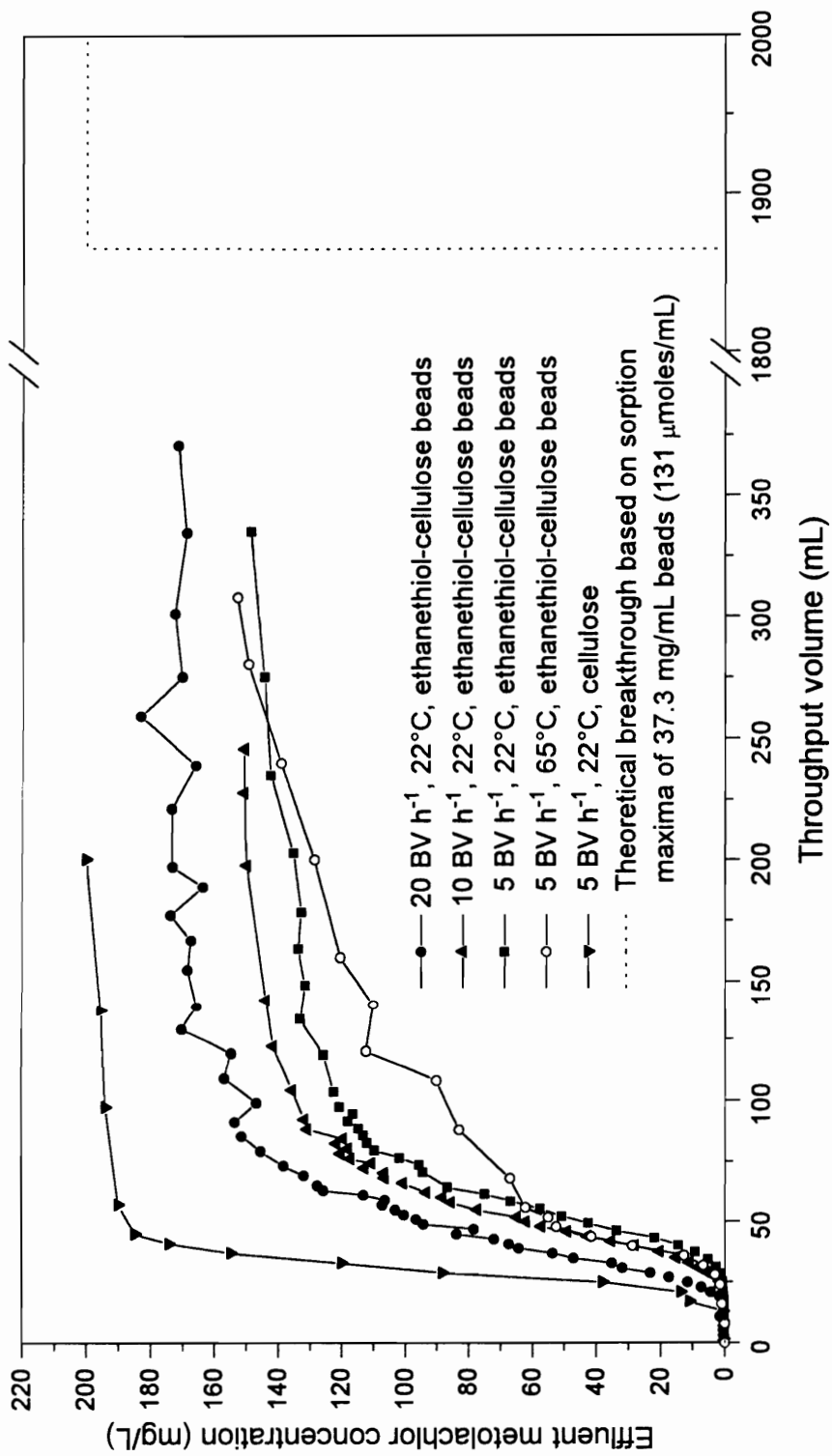


Fig. 5. Breakthrough curves for metolachlor sorption onto ethanethiol-cellulose beads in fixed bed column operations.

column, thus reducing the probability of a reaction between metolachlor and a thiol group.

Increasing temperature shifted the initial phase of the breakthrough curve slightly to the left, indicating a decreased physisorption (less retardation of metolachlor). At the same time, chemisorption of metolachlor increased, temporary, as indicated by the fact that the breakthrough curve leveled off at a lower effluent concentration. Higher temperatures may increase the kinetics of chemisorption.

The ethanethiol-cellulose beads were not able to maintain initial effluent quality over an extended time period or throughput volume, although a large capacity for metolachlor sorption was still available, as indicated by the continuous sorption at larger throughput volumes (> 75 mL). Initial effluent concentration may be improved by increasing pH and temperature, decreasing flow rate, and increasing bedvolume.

## CONCLUSION

Nonderivatized cellulose did not show any sorptive capacity towards metolachlor in batch sorption and fixed-bed column studies. Ethanethiol-cellulose did adsorb metolachlor. Sorption was a combination of mostly chemisorption and some physisorption. Higher temperatures and pH increased

sorption of metolachlor significantly. At pH 9 and 40 °C, a sorptive capacity of 58 mg per mL bedvolume was obtained under batch sorption conditions. Fixed-bed column studies showed that a large capacity for metolachlor was present on the ethanethiol-cellulose beads; however, high effluent quality was not maintained over a long period of time. Effluent quality may be improved by increasing pH, temperature, and bedvolume, and decreasing flow rate.

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## **CHAPTER V**

### **Carbofuran Degradation and Metabolite Incorporation During Solid State Fermentation**

## ABSTRACT

To address an urgent need for effective and economical methods to dispose of concentrated and dilute formulated pesticide or solutions such as waste rinsate, we investigated the suitability of using solid state fermentation (SSF) as a means to dispose of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) as Furadan 4F. The fate of [U-<sup>14</sup>C] ring labeled carbofuran was determined in incubation chambers containing either sphagnum peat moss or a peanut hulls:peat:steam-exploded wood (1:1:1) mixture (PPW). Ethyl acetate extractable, and alkali soluble and insoluble fractions were evaluated for radioactivity following either a four or eight week incubation period at 35°C. The majority of the radioactivity (80-98% of applied activity) was associated with the matrix materials at the end of both incubation periods with no significant mineralization observed. For peat, the majority of radioactivity was associated with the alkali insoluble fraction (63.5%), with only 8% of the applied activity found in the ethyl acetate extract after 8 weeks. On the other hand, for the PPW mix, 71% of the applied radioactivity was recovered in the ethyl acetate extract, with only 11% of the applied activity found in the alkali insoluble fraction after 8 weeks. The radioactivity contained in the alkali soluble fractions (fulvic and humic acid associated pesticide residues) of peat and PPW mix were similar after 4 and 8 weeks of incubation. With time, ethyl acetate

extractable radioactivity decreased, and alkali soluble and insoluble radioactivity increased in both SSF media. We contend that the ethyl acetate extracts contained mostly physisorbed pesticide residues, while the alkali soluble and insoluble fractions contain mainly incorporated (i.e., covalently bound or chemisorbed to the matrix) pesticide residue. The metabolite carbofuran-7-phenol, detected in the ethyl acetate extracts of the peat and PPW mix following 4 and 8 weeks of incubation, was susceptible to oxidative coupling in the presence of horseradish peroxidase and  $H_2O_2$ , suggesting that this metabolite may be incorporated into organic matter during humic substance formation. Our results showed that the rates of carbofuran residue incorporation were greater in the peat matrix compared to the PPW mix. These results may provide the basis for the development of an effective and economical method for detoxification or containment of waste pesticide residue.

## INTRODUCTION

Small-scale farm operators and pesticide applicators are in urgent need of methods to dispose of unused concentrated and dilute formulated pesticide wastewaters (e.g., rinsate). Improper disposal of pesticide containing wastewater is a potential hazard to soil, surface waters, and groundwater. Currently, the majority of the methods available for treatment of such waste solutions are either too costly, or involve complicated procedures or equipment (Norwood, 1990). Recently, Mullins et al. (1992a, 1992b) proposed a biologically-based system for treatment of pesticide-laden wastewater which included batch adsorption using lignocellulosic materials such as peat or steam exploded wood fiber, followed by separation (filtration), and finally degradation of the sorbed pesticide in bioreactors during solid state fermentation (SSF). The use of SSF as a means to dispose of pesticide residues has been investigated by Mullins et al. (1989), and Berry et al. (1993a,b). These authors reported significant decreases in the amount of solvent extractable pesticide residues (metolachlor, chlorpyrifos, atrazine, carbofuran, and diazinon) during SSF in bioreactors. Berry et al. (1993 a,b) suggested that the disappearance of pesticide resulted from both abiotic and microbial mediated degradation as well as from incorporation of the pesticide residue into organic matter. Alternatives for the final disposal of spent sorbent materials following bioreactor operation

include incineration, or possibly, land application. Before land application of spent sorbent can be considered a viable alternative, the fate of pesticides during SSF must be further evaluated. Several possibilities need to be considered, including: (1) mineralization, (2) transformation, (3) incorporation into and adsorption on organic matter and (4) volatilization.

We evaluated these possibilities using [U-<sup>14</sup>C] ring labeled carbofuran as the test compound and a benchtop SSF system similar to the one used by Petruska et al. (1985). While carbofuran, a carbamate insecticide, is readily degraded or transformed in soils (Arunachalam and Lakshmanan, 1990; Parkin and Shelton, 1992; Shelton and Parkin, 1991; Turco and Konopka, 1990; Getzin and Shanks, 1990; Singh and Sethunathan, 1992; Ou et al., 1982), several studies using <sup>14</sup>C ring labeled carbofuran showed no significant mineralization of the aromatic ring structure (Arunachalam and Lakshmanan, 1990; Turco and Konopka, 1990; Singh and Sethunathan, 1992; and Ou et al., 1982). Several authors (Arunachalam and Lakshmanan, 1990; Singh and Sethunathan, 1992) found carbofuran-7-phenol as the major metabolite.

Carbofuran is primarily degraded through the hydrolysis of the carbamate linkage, either chemical or enzymatically mediated, and is dependent on soil properties such as pH and moisture (Arunachalam and Lakshmanan, 1990; Parkin and Shelton, 1992; Shelton and Parkin, 1991; Pussemier et al., 1989). Although complete mineralization was not significant in soils, Chaudry and Ali

(1988) and Ramanand et al. (1988) were able to isolate bacteria from soil capable to completely mineralize carbofuran (including the aromatic ring) in enrichment cultures, using carbofuran as a sole C and N source. Ramanand et al. (1988) suggested that the microbially mediated hydrolysis of the carbamate linkage, was the first step in complete mineralization of carbofuran. Karns and Tomasek (1991) isolated a carbofuran hydrolase enzyme from carbofuran degrading bacteria. The work of Chaudry and Ali (1988) further showed the presence of bacteria that could only transform carbofuran to carbofuran-7-phenol.

An interesting explanation for the lack of, or low mineralization of the aromatic moiety of carbofuran in soils can be found in earlier work of Getzin (1973). The author added  $^{14}\text{C}$  ring labeled carbofuran-7-phenol to a silt and clay loam soil and found that after 2 weeks 70 to 80% of the radioactivity was unextractable. The formation of large amounts of non extractable, or soil bound residues was also reported by Turco and Konopka (1990) and Ou et al. (1982). Turco and Konopka (1990) suggested a two step mechanism for the fate of carbofuran in soils: (1) hydrolysis of the carbamate linkage, and (2) coupling of the primary metabolite, carbofuran-7-phenol, to soil. Organic matter content of soils has been implicated as major factor controlling adsorption and bound residue formation (Singh and Sethunathan, 1992; Singh et al., 1990; Sukop and Cogger, 1992).

Enzyme mediated incorporation of xenobiotics into organic matter has been described by Bollag and Loll (1983), and Berry and Boyd, (1985a). As pointed out by Bollag and Bollag (1989), enzyme-catalyzed polymerization and/or binding of pesticides to soil residues may represent an effective means of removing certain compounds from soil and water (Bollag and Bollag, 1989).

The objectives of this study were (1) to evaluate the fate of carbofuran during SSF with respect to volatilization, mineralization, transformation, and incorporation, in a sphagnum peat moss matrix and in a peanut hulls:peat:steam exploded wood (PPW) mix, and (2) to test the oxidative coupling potential of carbofuran and the metabolite carbofuran-7-phenol in the presence of H<sub>2</sub>O<sub>2</sub> and peroxidase.



## MATERIALS AND METHODS

**Chemicals.** Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 3-hydroxy carbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl methylcarbamate), 3-keto carbofuran (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl methylcarbamate), 3-keto 7-phenol (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol), carbofuran-7-phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol), 3-hydroxy 7-phenol (2,3-dihydro-2,2-dimethyl-3,7-benzofuranol), Furadan 4F (40,6 % a.i.) and [U-<sup>14</sup>C] ring labeled carbofuran (specific activity of 1.45 GBq/mmol) were donated by FMC Corp., Princeton, NJ. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was purchased from Aldrich Chemical Co., Inc. Milwaukee, WI. Horseradish peroxidase (HRP) type II having a specific activity of 200 purpurogallin units per mg was obtained from Sigma Chemical Co., St. Louis, MO.

**Incubation chamber setup and operation.** Twenty grams of corn meal, and 125 mL of a NPK solution (2 g/L Miracle-Gro), were added to 60 g of air dried and sieved (2 mm) sphagnum peat moss. A 15 mL bacterial inoculum was then mixed in with enough distilled water to bring the entire mix to a moisture content of 2.34 kg water per kg dry material. Following addition of 10 g crushed limestone the peat plus nutrient amendments were thoroughly mixed and allowed to equilibrate at room temperature ( $22 \pm 1^\circ\text{C}$ ) for 48 h. A 1:1:1

spaghnum peat, peanut hulls, and steam exploded wood mixture was prepared as described above, except that limestone was not added. The bacterial inoculum was obtained from a carbofuran degrading enrichment culture and consisted of a Gram-negative, motile, rod shaped aerobe capable of using carbofuran as the sole energy and carbon source through hydrolysis of the carbamate linkage forming carbofuran-7-phenol (Taraban, 1993). A stock solution of 3.7 MBq/1.5 mL  $^{14}\text{C}$  carbofuran in acetonitrile was prepared. A working solution was prepared by adding 1 mL of the stock solution to 240 mg Furadan 4F and stirred for one hour prior to the addition of 10 mL distilled water. Six hundred microliters of working solution, containing 128 kBq  $^{14}\text{C}$  carbofuran and 5.08 mg carbofuran, were added to 10 g portions of lignocellulosic materials subsequent to being placed in 50 mL incubation chambers. A total of eight incubation chambers were prepared per matrix material. Two of the eight chambers were placed in the freezer and served as a control. The others were incubated for 4 and 8 weeks at 35°C (three replicates per time period) in a  $\text{CO}_2$  collection apparatus (Petruska et al., 1985). At the end of the incubation period, the distribution of radioactivity in  $\text{CO}_2$ , volatiles, ethyl acetate extract, fulvic and humic acids, and alkali insoluble fraction was determined.

An overview of the complete fractionation scheme is shown in Figure 1. The incubation chambers were flushed with moist air for 1.5 minutes every

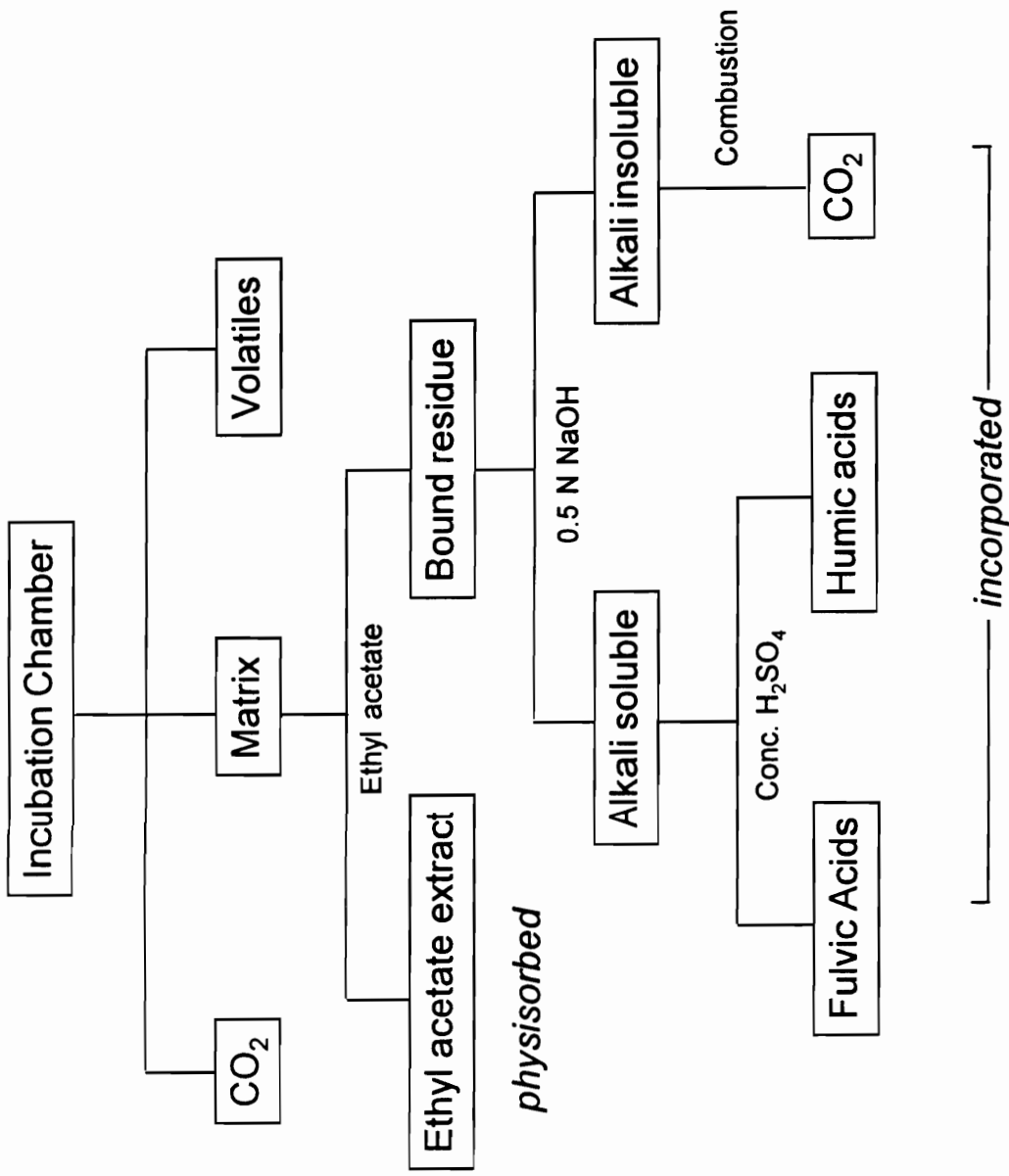


Figure 1. Distribution of radioactivity following solid state fermentation: fractionation scheme.

hour. The outgoing air was first passed through polyurethane foam plugs (three) to trap any volatiles and then bubbled through 3 mL of a 5N KOH solution to trap evolved CO<sub>2</sub>. The KOH solution was removed at regular intervals (2 to 4 days) and replaced by fresh KOH solution. Any <sup>14</sup>CO<sub>2</sub> present was trapped in 80% ethanolamine-coated scintillation vials by evolving CO<sub>2</sub> in the KOH solution with 25N H<sub>2</sub>SO<sub>4</sub> in a specially designed trapping device (Petruska et al., 1985). The evolved <sup>14</sup>CO<sub>2</sub> trapped in the 80% ethanolamine was quantitated by liquid scintillation counting (Beckman LS-3150 T scintillation counter) using Scintiverse BD universal L.S.C. cocktail (Fisher).

Volatile residues were trapped in three polyurethane plugs, which were replaced every 2 weeks. The plugs were placed in sealed flasks containing 25 mL methanol and extracted for 3 hours. Liquid scintillation counting (LSC) was used to analyze a 200µl aliquot of the methanol extract.

At the end of the incubation period, the solids were removed from the incubation chamber and placed in 50 mL Sorvall tubes. Forty milliliters of ethyl acetate was added and the mixture was homogenized using a Servall omni-mixer (Ivan Sorvall, Inc.) for one minute, followed by thirty minutes sonication and then allowed to stand for 12 hours at room temperature (22 ± 1°C). Solids were separated from the extracting solution by centrifugation (12,000 x g, 30 min). The extracting procedure was repeated an additional four times using sonication only (10 min). The amount of radioactivity contained in the combined

supernatants (referred to as ethyl acetate extracts) was determined by counting (LSC) a 200  $\mu$ l aliquot.

The remaining solids were extracted, using sonication (30 min), with 30 mL of 0.1N NaOH under N<sub>2</sub> atmosphere. After centrifugation at 12,000 x g for 30 min, the supernatant was removed and the procedure was repeated once. The amount of radioactivity contained in the combined extracts was measured by counting (LSC) a 200  $\mu$ l aliquot. Humic acids were precipitated from solution by adjusting the pH of the supernatant to 2 using 25N H<sub>2</sub>SO<sub>4</sub>. After 24 hours the humic acid fraction was separated from the solution (fulvic acid fraction) by centrifugation (12,000 x g, 15 min). The amount of radioactivity in the supernatant (fulvic acid fraction) was determined by LSC.

The solid material left behind following the alkali extraction procedure, was air dried and weighed prior to combustion of an aliquot using a Harvey biological oxidizer (Model OX500, R.J. Harvey Instruments, Corp.). The evolved CO<sub>2</sub> was trapped in a <sup>14</sup>C cocktail specially prepared by R.J. Harvey Instruments, Corp. for the biological oxidizer. The amount of evolved radioactivity was determined by LSC.

Thin Layer Chromatography (TLC) of ethyl acetate extracts was performed on 1000 $\mu$ m preparative Silica Gel G plates, using ether:hexane (5:1, v:v) as mobile phase. Amounts of 10  $\mu$ l were spotted. The plates were exposed to x-ray film for three weeks to visualize separated radioactive compounds.

Non-radioactive standards were visualized by iodine vapors. Radioactive compounds were removed from the plate and extracted from the silica with methylene chloride. The amount of radioactivity in the methylene chloride extracts was determined by LSC.

***Oxidative coupling experiments.*** The oxidative coupling experiments were conducted as described by Berry and Boyd (1985b). Coupling rates for ferulic acid, carbofuran, carbofuran-7-phenol, and a mixture of carbofuran-7-phenol and ferulic acid were determined. The reactions were carried out at room temperature ( $22 \pm 1^\circ\text{C}$ ) in 200 mL open beakers containing 100 mL phosphate buffered solution with  $\text{H}_2\text{O}_2$ , HRP, and phenolic compounds. The starting concentration levels of the reactants were 21.5  $\mu\text{moles}$  ferulic acid, carbofuran, and carbofuran-7-phenol, and 53.7 and 10.7  $\mu\text{moles}$  of ferulic acid and carbofuran-7-phenol, respectively for the mixture. HRP (1.52 a.u.) and 107.4  $\mu\text{moles}$   $\text{H}_2\text{O}_2$  were added to ferulic acid and the mixture, and 152 a.u. HRP and 10.74  $\mu\text{moles}$   $\text{H}_2\text{O}_2$  were added to carbofuran, and carbofuran-7-phenol. Five hundred microliter samples were taken at several time periods and added to 2 mL 50 mM KCN solution to stop the reaction. Samples were stored on ice in the dark until analysis. Ferulic acid, carbofuran-7-phenol, and carbofuran were analyzed by HPLC (LDC analytical system equipped with CM 4000 multiple solvent delivery system and 3100 variable wavelength detector). The mobile phase was  $\text{CH}_3\text{OH}:\text{H}_2\text{O}$  (70:30, v/v) for carbofuran and carbofuran-

7-phenol and acetonitrile:H<sub>2</sub>O:acetic acid (28.5:70:1.5, v/v) for ferulic acid. All samples were chromatographed over a 25 cm Whatman C-18 Partisphere column, and detected at 280 nm.

To reduce the possibility of enzyme destruction and inhibition, we were primarily concerned with initial stages of the coupling reactions (Berry and Boyd, 1985). The oxidative coupling rates were calculated using a zero order rate equation for the initial stages of the reaction:

$$\frac{dC}{dt} = -k$$

Where C is concentration of carbofuran-7-phenol, carbofuran, or ferulic acid in  $\mu\text{M}$ , and k is the reaction constant in  $\mu\text{M s}^{-1}$ .

## RESULTS

***Solid state fermentation.*** The total amount of radioactivity recovered was obtained by summing the activities from CO<sub>2</sub>, volatiles, ethyl acetate extracts, humic and fulvic acid extracts, and alkali insoluble fractions (Table 1). Recovery of radioactivity from the frozen controls for both matrix materials and incubation times was between 100 and 103% of the originally applied activity. The total amount of radioactivity recovered from the PPW mix were 98% and 103% after 4 and 8 weeks of incubation, respectively. Radioactive recoveries

**Table 1. Distribution of radioactivity following solid state fermentation**

Matrix	Wk	% recovery <sup>1</sup>								Total
		Ethyl acetate	Humic	Fulvic	Alkali insoluble	CO <sub>2</sub>	Volatiles			
Peat	4	29.03 (4.21) <sup>3</sup>	1.76 (0.82)	1.82 (0.45)	49.66 (6.34)	1.00 (0.33)	18.09 (4.29)			101.35 (2.39)
	8	8.28 (2.44)	2.45 (0.15)	11.36 (6.98)	63.49 (10.62)	1.35 (0.35)	19.40 (4.48)			106.33 (9.09)
Peat control	4	99.69	0.08	0.13	0.43	-	-			100.33
	8	100.47	0.08	0.24	0.57	-	-			101.36
PPW <sup>2</sup>	4	92.24 (2.47)	0.88 (0.32)	0.68 (0.08)	1.67 (0.70)	0.44 (0.04)	1.99 (0.23)			97.91 (1.45)
	8	73.74 (30.20)	2.56 (1.65)	9.94 (15.13)	11.42 (12.23)	0.71 (0.24)	3.49 (0.33)			101.86 (2.28)
PPW control	4	101.27	0.09	0.12	0.16	-	-			101.64
	8	102.81	0.1	0.18	0.24	-	-			103.33

<sup>1</sup> dpm measured/dpm initially applied x 100

<sup>2</sup> peanut hulls - peat - steam-exploded wood

<sup>3</sup> standard deviation



from the peat matrix were 101% and 106% after 4 and 8 weeks, respectively.

The total amount of radioactivity released as  $^{14}\text{CO}_2$  after 8 weeks of incubation from the PPW mix did not exceed 0.71% of the initially applied amount of radioactivity. A total of 1.35% of the initially applied amount of radioactivity was released as  $\text{CO}_2$  from the peat matrix (Table 1). The release of  $^{14}\text{CO}_2$  was a slow, steady ongoing process (Fig. 2).

Significant differences were observed between the amounts of radioactivity extracted from the polyurethane plugs recovered from the incubation chambers containing the two matrix materials. Radioactivity recovered as volatiles from the PPW mix was 1.99 and 3.49% after 4 and 8 weeks, respectively. This was low compared to the peat matrix, where 18.09 and 19.4% of the initially applied radioactivity was recovered as volatiles after 4 and 8 weeks, respectively (Table 1).

The results of further fractionation of matrix associated radioactivity are shown in Table 1, and Figures 3 and 4. For the frozen controls of both matrix materials at both the 4 and 8 week incubation times, essentially all (> 99%) radioactivity was recovered in the ethyl acetate extract. When comparing the two matrix materials, there was a large difference in the amount of ethyl acetate extractable radioactivity following SSF. Ethyl acetate extractable activity from the PPW mix was 92.24% after 4 weeks and 73.74% after 8 weeks of incubation. Much lower ethyl acetate extractable activity was found in the SSF

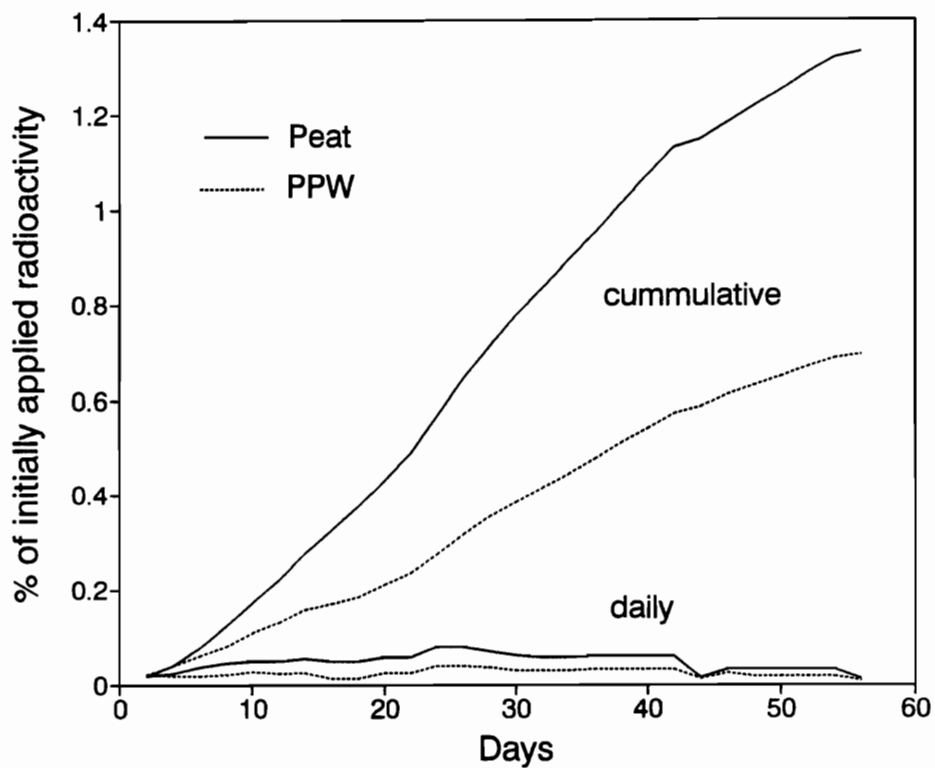


Figure 2.  $^{14}\text{CO}_2$  production during solid state fermentation of uniformly ring labeled carbofuran in a peat and peanut hulls - peat - steam-exploded wood (PPW) matrix.

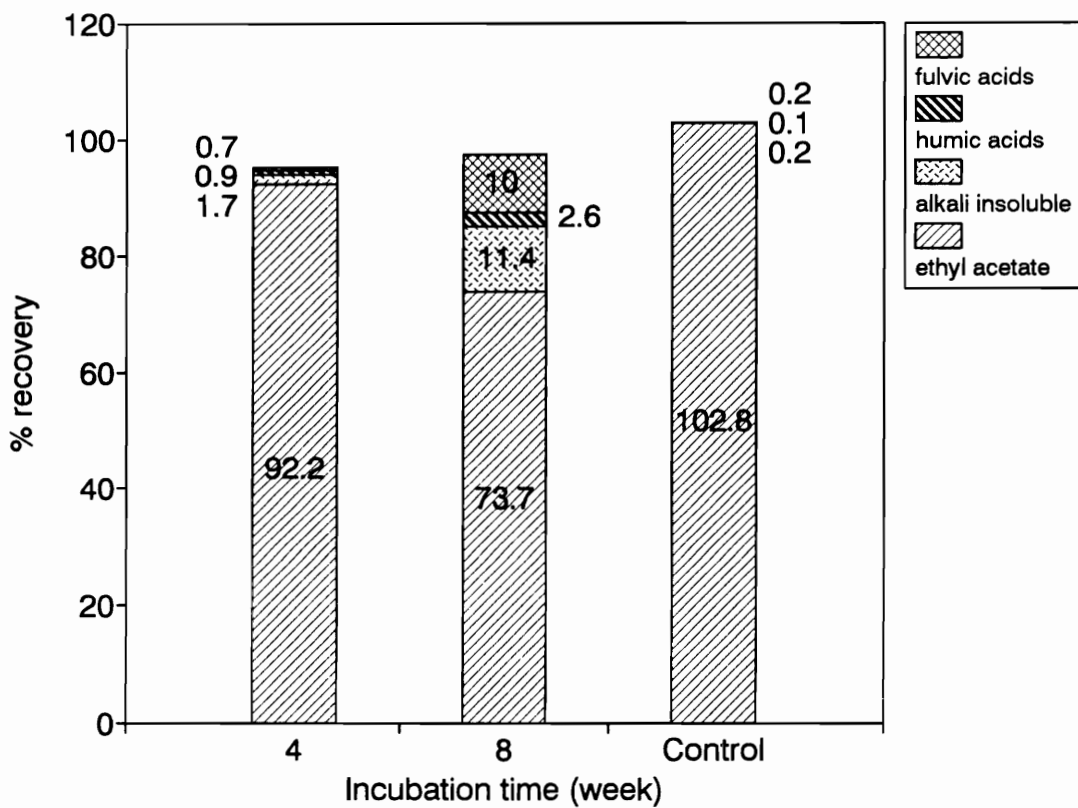


Figure 3. Distribution of matrix associated radioactivity in peanut hulls - peat - steam-exploded wood matrix following solid state fermentation.

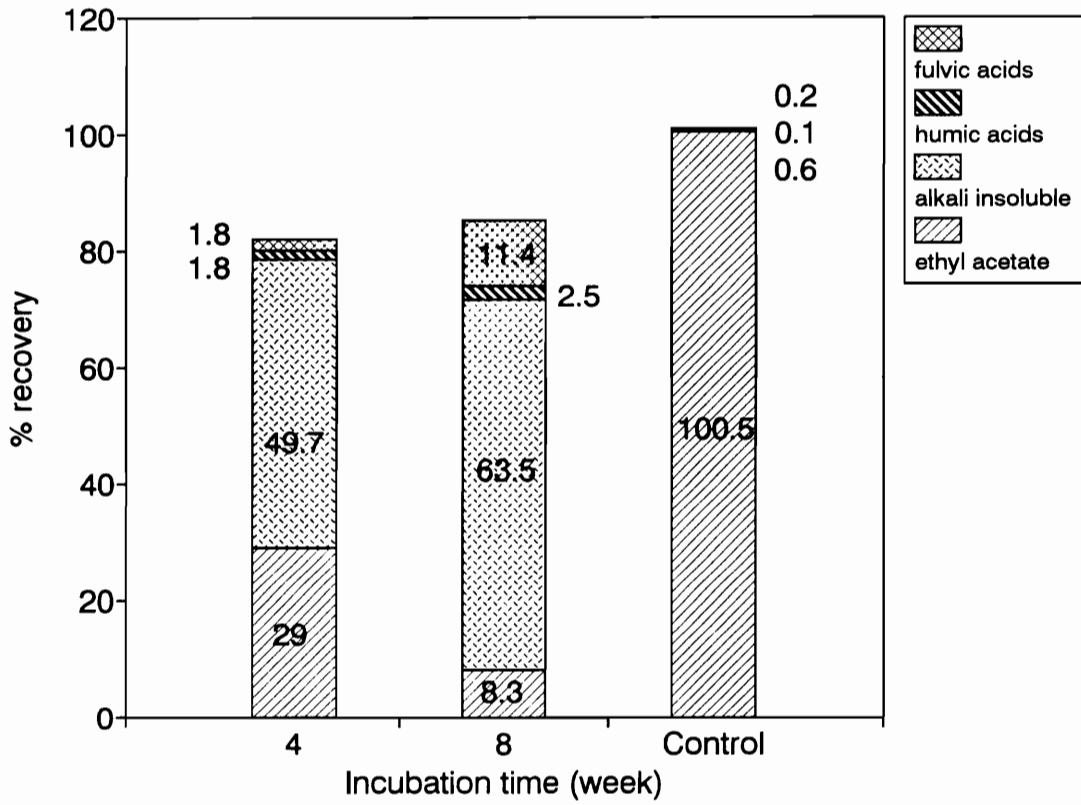


Figure 4. Distribution of matrix associated radioactivity in peat matrix following solid state fermentation process.

medium based on peat alone: 29.03% after 4 weeks and only 8.28% after 8 weeks of incubation.

The bound residue fractions include alkali soluble fractions (fulvic and humic acid fractions), and alkali insoluble fractions (Fig. 1). No significant amount ( $< 0.24\%$ ) of radioactivity was associated with humic and fulvic acids extracted from the lignocellulosic materials contained in the control chambers. In chambers containing the PPW mix, 0.88% and 0.68% of the initially applied radioactivity was associated with humic and fulvic acids, respectively, following 4 weeks of incubation. After 8 weeks, 2.56% and 9.94% of initially applied radioactivity was associated with humic and fulvic acids, respectively. For the peat matrix, similar results were obtained. The amount of radioactivity associated with fulvic acids increased from 1.82% to 11.36% from week 4 to week 8, while the radioactivity associated with humic acids increased from 1.76% to 2.45%.

The alkali insoluble associated radioactivity was between 0.43 and 0.57% for the control chambers containing peat, and between 0.16 and 0.24% for the PPW mix controls after 4 and 8 weeks, respectively. The highest levels of radioactivity associated with the alkali insoluble fraction were found for the peat matrix (49.66% after 4 weeks, and 63.49% after 8 weeks). For the PPW mix, only 1.57% and 11.42% of the initially applied radioactivity was associated with the alkali insoluble fraction after 4 and 8 weeks of incubation time,

respectively.

TLC of ethyl acetate extracts for both matrix materials indicated the presence of the parent compound, carbofuran ( $R_f = 0.58$ ), and one major metabolite, carbofuran-7-phenol ( $R_f = 0.77$ ), following 4 and 8 weeks of incubation. No other metabolites were detected. The relative distribution of the parent compound and the metabolite was different for the two matrix materials (Table 2). Carbofuran accounted for 94.9% and 93.32% (87.54% and 68.81% respectively, of the initially applied radioactivity) of the radioactivity in the ethyl acetate extract from the PPW mix after 4 and 8 weeks, respectively.

Carbofuran-7-phenol accounted for only 0.9% and 1.3% (0.83% and 0.96%, respectively, of the initially applied radioactivity) of the ethyl acetate extractable radioactivity after 4 and 8 weeks, respectively. The amount of radioactivity associated with the parent compound in the ethyl acetate extract of the peat matrix was 79.6% and 50.8% (23.11% and 4.21% respectively, of the initially added radioactivity) after 4 and 8 weeks, respectively. Carbofuran-7-phenol associated radioactivity in the ethyl acetate extract of the peat matrix increased from 9.11% after 4 weeks to 37.4% after 8 weeks (2.64% and 3.10% respectively, of the initially added radioactivity). Radioactivity of the ethyl acetate extracts of all controls was almost completely associated with the parent compound ( $\geq 97\%$ ).

**Table 2. Carbofuran and carbofuran-7-phenol associated radioactivity in ethyl acetate extract**

Matrix	Wk	% in ethyl acetate extract	
		Carbofuran	Carbofuran-7-phenol
Peat	4	79.6	9.1
	8	50.8	37.4
Peat control	4	97.1	0.6
	8	97.0	0.8
PPW <sup>1</sup>	4	94.9	0.9
	8	93.3	1.3
PPW control	4	97.1	0.7
	8	96.8	0.5

<sup>1</sup> peanut hulls - peat - steam exploded wood

**Oxidative coupling experiment.** While the parent compound, carbofuran, could not be oxidatively coupled in the presence of H<sub>2</sub>O<sub>2</sub> and horseradish peroxidase (152 a.u.), carbofuran-7-phenol was oxidatively coupled at a rate of 0.017  $\mu\text{M s}^{-1}$  in the presence of 152 a.u. horseradish peroxidase (Fig. 5). The coupling rate of carbofuran-7-phenol was greatly enhanced in the presence of ferulic acid (0.22  $\mu\text{M s}^{-1}$  at 1.52 a.u.). Ferulic acid, a natural occurring precursor for humic substance synthesis, coupled at the highest rate (2.84  $\mu\text{M s}^{-1}$  at 1.52 a.u.).

## DISCUSSION

The fate of carbofuran in soils has been studied by several authors (Arunachalam and Lakshmanan, 1990; Turco and Konopka, 1990; Singh and Sethunathan, 1992; and Ou et al., 1982). All these authors reported that carbofuran was readily degraded in soils, however, no significant mineralization of the aromatic ring structure was observed. In the present study, similar results were obtained, i.e., no significant mineralization of the aromatic ring of carbofuran during SSF in either matrix was observed. The disappearance rate of carbofuran in our peat containing incubation chambers was slightly more rapid than the rates reported by Berry et al. (1993b), whose studies were conducted in large scale bioreactors. The increased disappearance rate



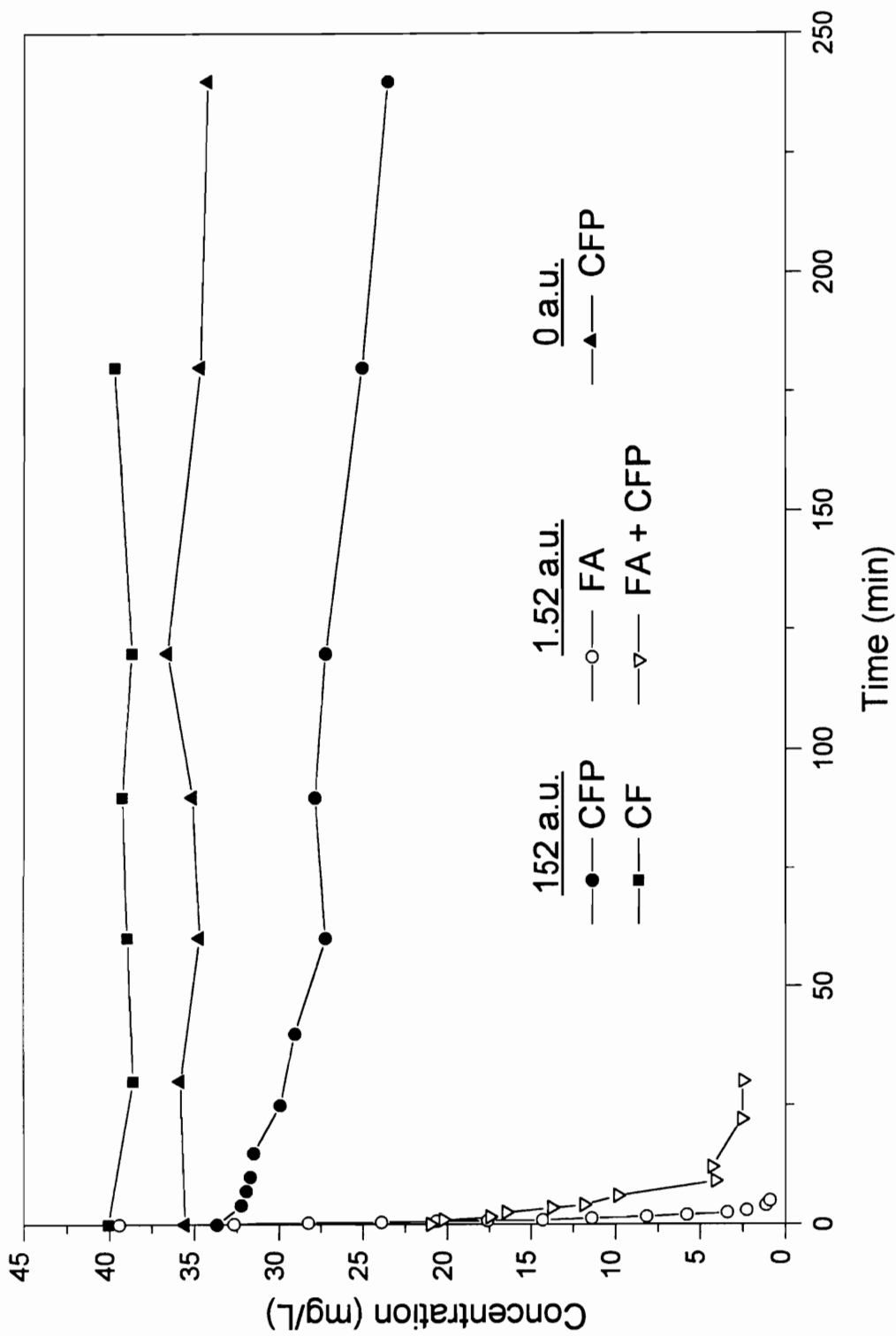


Figure 5. Oxidative coupling of carbofuran (CF), carbofuran-7-phenol (CFP), carbofuran-7-phenol and ferulic acid (CFP + FA), and ferulic acid (FA) by horseradish peroxidase (a.u. activity units).

observed in our study was most likely the result of consistently higher incubation temperatures (35 °C versus 26 °C).

The majority (80-98%) of the applied radioactivity was associated with the matrix at the end of the SSF process. The remaining portion was lost from the matrix through volatilization. Volatilization was highest in chambers containing the peat matrix. This may have resulted from the formation of a more volatile metabolite, or higher temperatures caused by greater microbial activity in the peat matrix (pH 5.6). The microbial activity in the PPW mix may have been reduced by the low pH in the PPW mix (pH 4). Thus, matrix associated activity is defined as all radioactivity except CO<sub>2</sub> and volatile fractions.

Undoubtedly, some of the pesticide residue and its transformation products were sorbed tightly to the matrix through van der Waals forces (physisorption). It is also likely that some, if not the major portion of the pesticide residues, were incorporated through covalent bond formation (chemisorption). While the ethyl acetate fraction includes primarily physisorbed pesticide and transformation products, a minor amount of incorporated pesticide residues was also likely included in this fraction because of the solubilization of some of the humic substances. We contend that a large portion of the pesticide residue associated with the alkali soluble (humic and fulvic acids), and alkali insoluble fractions is covalently bound to, or incorporated into organic matter. With time, an increase in alkali soluble and insoluble fractions, and a decrease in ethyl acetate

extractable fractions was observed, as more pesticide residue became incorporated.

As suggested above, the alkali soluble and insoluble fractions primarily contain covalently bound pesticide residues. Two mechanisms for covalent bond formation between pesticide residues and a lignocellulosic matrix can be envisioned: (1) enzymatic mediated oxidative coupling and (2) a direct chemical reaction (e.g. nucleophilic substitution). The extent of incorporation depends on reactivity of pesticide residues, reactivity of matrix (e.g., number of reactive sites), reaction environment, microbial (enzymatic) activity, and time. Covalent bond formation by nucleophilic substitution is likely of less importance for carbofuran and carbofuran-7-phenol, because the lack of an electrophilic center on the pesticide residues. However, results of the oxidative coupling experiments indicated that carbofuran-7-phenol did undergo oxidative coupling whereas carbofuran did not. Therefore, incorporation of carbofuran residues into organic matter most likely is the result of enzyme mediated oxidative coupling of the hydrolyzed residue (Fig. 6). Oxidative coupling is a generally accepted mechanism for the incorporation of phenols and aromatic amines into organic matter (Bollag and Loll, 1983). This suggests that the formation of carbofuran-7-phenol is essential before incorporation of carbofuran residues through oxidative coupling can occur. Earlier work by Turco and Konopka (1990) and Getzin (1973), also suggested that carbofuran-7-phenol was incorporated and

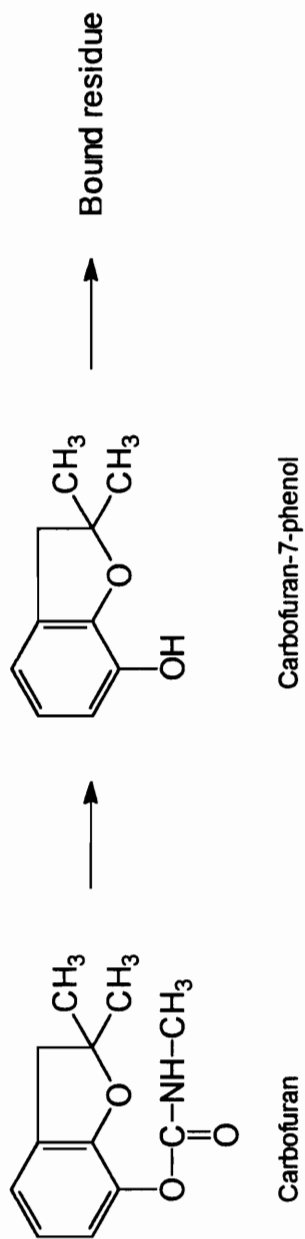


Figure 6. Fate of carbofuran during solid state fermentation in a peat matrix.

not the parent compound. The oxidative coupling rate of carbofuran-7-phenol, in the presence of ferulic acid, was of the same order of magnitude as coupling rates for guaiacol, catechol, and pyrogallol, which have been identified as naturally occurring phenolic humus constituents (Berry and Boyd, 1985b).

The absence of pesticide residue incorporation in the frozen controls indicates the importance of microbial activity. Microorganisms play an important role in hydrolysis of the carbamate linkage (Pussemier et al., 1989) and the production of peroxidases, a key enzyme in the oxidative coupling process. The lower rates of carbofuran residue incorporation in the PPW mix, as indicated by the low amounts of alkali soluble and insoluble activity, can be explained by differences in matrix reactivity and reaction environment. The PPW mix contained more lignin compared to peat which contained more humic substances. The lower amount of reactive functional groups, especially -OH, in lignin compared to humic substances (Stevenson, 1982) likely reduced the probability for incorporation. The pH of the matrix may also have played a key role. The low pH observed for the PPW mix probably reduced microbial activity, resulting in a decrease of microbial mediated hydrolysis of the carbamate linkage. This was also indicated by the absence of carbofuran-7-phenol in the ethyl acetate extract of the PPW mix (Table 2). Thus, the low rates of carbofuran residue incorporation in the PPW mix during SSF may have resulted from reduced formation of the metabolite carbofuran-7-phenol.

When disposal of the spent organic matrix is considered (e.g. land application) the potential hazard of the bound pesticide residue must be evaluated. We suggest that the ethyl acetate extractable pesticide residues (physisorbed) are most likely to be leached or represent a bioavailable component. In this regard, our results indicate that peat alone is a more desirable SSF medium than the PPW mix. In an overview, Bollag and Bollag (1989) stated that, to date, all available data indicate that release of bound residues is minimal, suggesting that once bound to humus, xenobiotics are unlikely to adversely affect the environment. This was further confirmed by later work by Dec et al. (1990) and Berry et al. (1993). Berry et al. (1993) showed that the leachability and bioavailability of carbofuran were minimal after a one year incubation period in peat filled bioreactors. Immobilization of pesticide residues through incorporation into organic matter during SSF in a lignocellulosic matrix such as peat, should not represent a hazardous situation if land application of the spent organic matrix is considered.

## CONCLUSION

Regardless of the lignocellulosic materials used, carbofuran did not undergo mineralization to any significant extent. The ethyl acetate extractable pesticide residue was made up of carbofuran and carbofuran-7-phenol, with the

latter being more abundant in the extract of the peat matrix. With time (for both SSF media), less pesticide residue was ethyl acetate extractable, and more pesticide residue became associated with the alkali soluble and insoluble fractions (incorporated). Pesticide residue associated with the alkali soluble and insoluble fraction was much larger ( $\approx 6$  times) in the peat matrix compared to the PPW mix. This was caused by differences in matrix reactivity, reaction environment and microbial activity. Our results further suggest that the formation of carbofuran-7-phenol likely plays a key role in the incorporation of carbofuran residues into organic matter. This evidence points to the importance of the reaction environment and microbial activity. Therefore, we contend that the SSF process involving peat (8 weeks) reduces leaching potential and bioavailability of carbofuran pesticide residues to a larger extent than the SSF process based on the PPW mix. We believe that hazards associated with the incorporated (i.e. covalently bound) pesticide residues will be low; however, it will be necessary to conduct long term fate studies to investigate possible release of incorporated residues during organic matter turnover in soils.

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## **CHAPTER VI**

### **Summary and Conclusion**

Three Ambersorb adsorbents were compared with Filtrasorb 400 (activated carbon) for adsorption of formulated metolachlor (Dual 8E) and dicamba (Banvel 4L) from aqueous solutions. Ambersorb 572 adsorbent was superior to Filtrasorb 400 in fixed-bed adsorption columns because of its greater adsorptive capacity and good regenerability characteristics. Superior adsorptive capacity of Ambersorb 572 adsorbent was the result of a favorable pore size distribution, improving mass transfer kinetics in flow through systems. Regardless of the adsorbent used, fixed-bed columns were more effective in the cleanup of metolachlor as Dual 8E containing water compared to dicamba as Banvel 4L containing water.

Immobilization of pesticides through covalent bond formation on a solid support may be an efficient means of decontaminating pesticide containing water. Especially immobilization of electrophilic pesticides (e.g. atrazine, simazine, alachlor, and metolachlor) through nucleophilic substitution reactions may prove successful. Ethanethiol-cellulose beads were synthesized by a novel method, reacting carbonyldiimidazole activated beads with aminoethanethiol. Thiol contents of up to 160  $\mu\text{moles SH/mL}$  beads were obtained. Sorption of metolachlor by ethanethiol-cellulose beads increased at elevated pH and temperatures, and sorptive capacities were similar to Ambersorb 572 adsorbent in batch studies. Sorption of metolachlor by ethanethiol beads was primarily the

result of chemisorption, with some physisorption occurring. Occurrence of both sorption mechanisms was further confirmed by fixed-bed column studies.

The fate of carbofuran during solid state fermentation in a peat matrix and a peanut hulls - peat - steam-exploded wood (PPW) matrix was investigated in small bioreactors. Solid state fermentation may prove an effective means to dispose of pesticide regenerate or spent lignocellulosic adsorbents. Up to 77.5 % of carbofuran residue was incorporated (immobilized) into organic matter following 8 weeks of incubation in a peat matrix. Only 24 % of carbofuran residue was incorporated in PPW matrix. Transformation of carbofuran to carbofuran-7-phenol is the initial step in the incorporation of carbofuran residues into organic matter. Incorporation is likely the result of peroxidase mediated oxidative coupling reactions. As a result of incorporation, pesticide residues may become permanently detoxified.

Pollution of groundwater and surface water as the result of improper handling and disposal of pesticide waste calls for on site waste treatment systems. A treatment system based on sorption technology consists of two phases, a sorption step followed by regeneration of the adsorbent and disposal of the pesticide regenerate, or disposal of the spent adsorbent in case a non regenerable sorbent is used. Ambersorb 572 adsorbent may prove useful as a

regenerable sorbent. The pesticide regenerate could be added to bioreactors for final disposal. Ethanethiol-cellulose beads can be used as sorbents for electrophilic pesticides in batch adsorption systems. It may also be used as a second sorbent following a general sorbent, to reduce pesticide concentrations to lower ppb levels. Spent ethanethiol-cellulose beads can be added to bioreactors as such. Solid state fermentation in bioreactors may be useful as a means of final disposal for pesticide residues, provided that the pesticide or its transformation products can be incorporated into organic matter, or that the pesticide is completely mineralized. Phenolic and anilinic residues are likely susceptible to incorporation into organic matter. This research, in addition to other research of the Pesticide Waste Disposal Group, proved the feasibility of the proposed on site treatment strategy. Future research should be focused on the combination and optimization of several aspects of the system.

## VITA

Hans Willems was born on October 30th, 1967, in Ghent, Belgium. He lived the first nine years of his life in Rotterdam (The Netherlands). When his parents decided to move back to Belgium, they choose Sint-Niklaas, a provincial town in the heart of "Het land van Waas". There he went to high school at the "Sint-Jozef-Klein-Seminarie", where he selected "Latin-Mathematics" as major. Most of his free time, until 1990, was spent as a boy scout leader in Sint-Joris, a local boy scout group. He graduated from high school in june 1985. Three months later he started college at the State University of Ghent. Being interested in exact sciences, and more specific, in the environment, he decided to study at the College of Agronomy (recently renamed as the College of Applied Biological Sciences). He completed this five year program with a thesis study on the "Chemical Behavior of Boron in Soils and Uptake by Plants". He graduated in June 1990 as "Engineer in Chemistry and Agricultural Industries" with great distinction. After some thoughts, he decided to start a Ph.D program in the Department of Crop and Soil Environmental Sciences at Virginia Polytechnic Institute and State University in Blacksburg. During four years he worked on the "Development of Sorption Technology for the Cleanup of Pesticide Contaminated Wastewater". He graduated from Virginia Tech in December, 1994.

