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Abstract

Title of Thesis: Biodegradation of Hexachlorocyclohexane in Sand and Wastewater Using Immobilized *Phanerochaete chrysosporium*

by

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Masters of Science in Chemical Engineering (1990)

Thesis directed by

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and

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The present study was carried out in order to assess the ability of *Phanerochaete chrysosporium* to degrade lindane (hexachlorocyclohexane) in sand as well as in water. It was found that the fungus is immobilized on saw dust, it is able to degrade lindane in a packed-bed reactor configuration, with a first-order rate constant of $0.00066 min^{-1}$. The fungus is also able to degrade lindane in sand. As with the packed-bed reactor, a supplemental carbon source (glucose) is required. In this study it was found that a glucose concentration of 20 gm/kg of sand, and 40% w/w moisture content, give the highest rate of degradation, with a first-order rate constant of $0.105\ day^{-1}$.

BIODEGRADATION OF HEXACHLOROCYCLOHEXANE IN SAND AND WASTEWATER USING IMMOBILIZED PHANEROCHAETE CHRYSOSPORIUM

by

Ajaykumar C. Gami

Thesis submitted to the Faculty of the Graduate School
of the New Jersey Institute of Technology in partial
fulfillment of the requirements for the degree of
Masters of Science in Chemical Engineering
1990

Approval Sheet

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Chapter 1

Introduction

Many toxic or carcinogenic organohalides persist in the environment and tend to accumulate in the body fat of animals occupying higher trophic levels (Bumpus et al. 1985). One reason of persistence in the environment of these compounds is that microorganisms are either unable to degrade them or do so very slowly.

Lignin is a naturally occuring highly complex heteropolymer, which is resistant to attack by most organisms. Its biodegradation is thought to be the rate limiting step in the carbon cycle (Bumpus et al. 1985). *Phanerochaete chrysosporium*, a common wood rot fungus, secretes a unique hydrogen peroxide-dependent, extracellular, lignin-degrading enzyme system which is capable of breaking down lignin, a complex aromatic polymer that is otherwise very resistant to decay. The ability of this fungus to degrade lignin suggested that recalcitrant organohalides having a very stable benzene nucleus may also be degraded by this organism.

Several recent papers have described the ability of this fungus to mineralize a wide spectrum of chlorocarbons, such as DDT, polychlorinated biphenyls, polychlorinated dibenzo(p)dioxins, chlorinated alkanes and chlorophenols (Bumpus et al. 1985, Faison et al. 1985, Lewandowski et al. 1988,

Tien et al. 1984). It appears that this fungus holds a great deal of interest as a biological treatment tool in hazardous waste management.

Many studies have been carried out to investigate the feasibility of using *Phanerochaete chrysosporium* for bioremediation of selected xenobiotics in liquid cultures (Bumpus et al. 1985, 1989 Eaton 1985). However, the ability of this fungus to degrade recalcitrant xenobiotics in soil or sand has received much less attention. Information on the ability of these organisms to degrade toxic compounds, such as lindane, to innocuous products in sand and soil is necessary before the organism can be considered for use in site bioremediation. Lindane (1,2,3,4,5,6 hexachlorocyclohexane) has been extensively used as a bactericide, herbicide, algicide and insecticide. Therefore, the purpose of this research work was to demonstrate the capability of the fungus to biodegrade lindane in sand and wastewater.

Chapter 2

Literature Review

2.1 Degradation in Wastewater

The wood rot fungus *Phanerochaete chrysosporium* has been the focus of much scientific interest in recent years. The organism was found to produce an extracellular enzyme system associated with important degradation reactions. Various authors have used the terms "ligninase" (Tien et al. 1984) and "diarylpropane oxygenase" (Glenn et al. 1983) in describing the lignin degrading enzyme system. Each of these terms is intended to describe a class of isoenzymes requiring hydrogen peroxide for activity. These enzymes are non-specific and they are found to degrade various chlorinated hydrocarbons.

Leatham et al. (1983) studied POL88, a mutant strain of *Phanerochaete chrysosporium*, and found that it can degrade different aromatic compounds. Thirty-six aromatic compounds were tested for degradability by ligninolytic cultures. Sixteen compounds were degraded by at least 20% in three days. These investigators obtained high rates of degradation for organic acids and low rates of degradation for phenolic compounds. They concluded that this fungus was among the most versatile and non-specific degraders of aromatic molecules examined.

Arjmand et al. (1985) studied the degradation of 4-chloroaniline and 3-4-dichloroanilines, and obtained 35% and 50% mineralization, respectively. Several fungal chloroaniline-derived metabolites were formed, but the free chloroanilines and their azo and azoxy derivatives could not be detected. Hence, they concluded that complete removal of chloroanilines took place.

Bumpus et al. (1985) studied the degradation of DDT, 3,4,3'4'-tetrachlorobiphenyl and 1,2,3,4,5,6-hexachlorocyclohexane in suspended cultures. They found that these compounds are completely mineralized to CO_2. Model studies, based on the use of DDT suggested that the ability of *Phanerochaete chrysosporium* to metabolize these compounds is dependent on the extracellular lignin-degrading enzyme system of the fungus.

Eaton (1985) tested *Phanerochaete chrysosporium* and several other different microorganisms for mineralization of PCB. Among the five microorganisms tested for the degradation of arochlor. *(Phanerochaete chrysosporium, Phlebia brevispora, Funalia gallica, Coriolus versicolor and Poria cinerescens) Phanerochaete chrysosporium* mineralized the most arochlor and was chosen for detailed study. Eaton also suggested that PCB degradation begins at the onset of secondary metabolism, triggered by nitrogen limitation. He found that in those cultures in which the carbon/energy source had been depleted, mineralization of PCB ceased. This suggested that PCB was not used by the fungus as a primary carbon source.

Pellinen et al. (1988) studied the dechlorination of high-molecular-weight chlorolignin. They found that the total organic chlorine content of chlorolignin decreased by almost 50% during one day of treatment, and a

corresponding amount of inorganic chloride was liberated into the solution. They suggested that the process is an enzyme-initiated (lignin peroxidase) auto-oxidation.

Phanerochaete chrysosporium was used by Mileski et al. (1988) for the degradation of pentachlorophenol (PCP). They found that the time of onset, time course, and eventual decline in the rate of PCP mineralization were similar to those observed for lignin degradation. This led them to conclude that the lignin-degrading system of this fungus is involved in the degradation of PCP.

The ability of *Phanerochaete chrysosporium* to degrade polycyclic aromatic hydrocarbons (PAH) that are present in anthracene oil has also been demonstrated (Bumpus et al. 1989). They showed that at least twenty-two different PAHs, including all of the most abundant PAH components present in anthracene oil, underwent 70 to 100% disappearance during 27 days of incubation with nitrogen-limited cultures of this fungus.

Phanerochaete chrysosporium was used by Huyuh et al. (1985) to degrade chlorinated organics in waste water treatment systems. Most of the chlorinated phenols and other low molecular weight components and their chlorinated derivatives were removed. Veratryl alcohol was the major end product. It was concluded that the degradation mechanism involved methylation, oxidation and reduction.

Sanglard et al. (1986) showed that *Phanerochaete chrysosporium* rapidly oxidized benzopyrene (a polyaromatic hydrocarbon) to CO_2. Some 99.5% of benzopyrene was converted to metabolic products in 54 hours. Extracellular

ligninase was shown to be involved in the initial oxidation reaction. It was concluded that the ligninase is non-specific and non-stereo-selective.

Pak (1988) found that *Phanerochaete chrysosporium* can degrade 2-chlorophenol effectively. In his experiment with shaker flasks, he was able to degrade 20 ppm of 2-chlorophenol in less than four hours. He was able to show fungal degradation activity in packed as well as fluidized bed reactor experiments. He analyzed various types of reactor configurations and came to the conclusion that a packed bed reactor with porous silica beads as a inert support was the optimal configuration.

Haq (1989) showed that *Phanerochaete chrysosporium* is not active when grown in suspension in a well agitated fermenter. However, the degradative activity may be increased by allowing the fungus to grow on and into porous support particles in the agitated fermenter. He showed that wood chips can be used instead of porous silica beads in the packed bed reactor without losing any fungal degradative activity. He also used H_2O_2 in the feed media and showed that the addition of H_2O_2 not only results in an increase in the fungal degradative activity, but also acts as an alternative source of oxygen during the lignolytic enzyme production period.

In another study, Zitzelsberger et al. (1987) reported that *Phanerochaete chrysosporium* was able to degrade veratrylglycerol-2,4 dichlorophenyl ether, a lignin bound xenobiotic residue.

2.1.1 PROCESS PARAMETERS AFFECTING THE BIODEGRADATION PROCESS IN WASTEWATER

The effect of process parameters on the activity of the enzyme system is not well defined since very little knowledge about the aromatic cleavage is available. However, the degradation of lignin has been studied extensively, and it is believed that degradation of chlorinated aromatics follows a path similar to that of lignin degradation (Haq, 1989). Various parameters for degradation of lignin have been studied extensively, and their effect is reported below.

2.1.1.1 Effect of Nitrogen

The concentration of nitrogen in the medium is found to be very critical for lignolyitc activity (Bumpus et al. 1990,1985; Eaton 1985; Faison et al. 1985, 1986; Kirk et al. 1986; Reid et al. 1983). Reid et al. (1983) studied the effect of nitrogen sources and supplements on degradation of aspen wood lignin. He monitored the growth of *Phanerochaete chrysosporium* on cellulose and suggested that the addition of nitrogen strongly inhibits lignin degradation, but the addition of complex nitrogen sources such as peptone, albumin, casein-hydrolysate and yeast extract can stimulate the rate of degradation.

Kirk et al. (1986) showed that the source of nutrient nitrogen (NO_3^-, NH_4^+, amino acids) had little influence on lignin degradation, but the concentration of nitrogen was critical. The rate of lignolytic activity was only 25-35% in a medium containing 24mM of nitrogen than in one containing 2.4mM of nitrogen. The mechanism for the adverse effect of high nitrogen concentration on lignin metabolism is not known.

Faison et al. (1985) detected higher ligninase 24 to 36 hours after the end of the active growth phase. They found that additions of either glutamate or NH_4^+ to nitrogen-starved cultures suppresses ligninase activity.

2.1.1.2 Effect of Oxygen

The concentration of oxygen was also found to be an important rate limiting factor, since lignin degradation has been shown to be an oxidative process. Kirk et al. (1986) examined lignin metabolism as a function of oxygen concentration. They maintained cultures under a one-atmosphere gas phase that contained 5%, 21% or 100% oxygen. Although the growth during the first three days of incubation was similar at all three O_2 concentrations, lignin decomposition began after the initial growth period under 21% and 100% O_2, but not under 5% O_2.

2.1.1.3 Effect of pH

The effect of the pH of the medium was also found to be an important factor for ligninase activity. Kirk et al. (1986) found that the optimum pH for degradation of lignin is 4.5, and substantial reduction in activity takes place for pH values lower than 3.5 and above 5.5. However, they found the optimum pH for growth to be 5.5.

2.1.1.4 Effect of Carbon Source

Kirk et al. (1986) suggested that a defined medium composition is required in order to get optimum growth and ligninase activity. They demonstrated that any of several carbohydrates can serve as growth substrates supporting lignin metabolism. Bumpus et al. (1990) showed that mineralization of xenobiotics ceased after 20-25 days of incubation in cultures in which simple carbohydrates served as a

carbon source. However, sustained rates of xenobiotic mineralization were achieved for at least 90 days when complex carbohydrates (i.e., cellulose and starch) were used as a carbon source. Reid et al. (1983) showed that cultures whose carbon/energy source had been depleted stop mineralizing.

2.1.1.5 Effect of Agitation

The effect of agitation on the ligninase activity has also been studied (Faison et al. 1985; Jager et al. 1985; Leisola et al. 1985; Reid 1983). Most of the studies carried out in suspended cultures showed that agitation suppresses ligninase activity. Reid et al. (1985) showed that cultures agitated in a gyratory shaker degraded lignin to carbon dioxide as effectively as static cultures. However, Pak (1988) found that the fungus is not particularly active in a suspended growth reactor and needs to be attached to a surface.

Leisola et al. (1985) reported that the extracellular H_2O_2-dependent ligninase activity of *Phanerochaete chrysosporium* was produced in agitated cultures when veratryl alcohol or veratraldehyde were added to the cultures. Too high an agitation speed led to complete inhibition of both the reduction reaction and the ligninolytic activity.

Jager et al. (1985) examined the effects of detergents on development of ligninase activity in agitated cultures. They showed that addition of Tween-80, Tween-20 or 3-1-propanesulfonate to the cultures permits development of ligninase activity comparable to that routinely obtained in stationary cultures. They suggested that the detergents probably supplied fatty acids to the cultures.

In order to overcome the sensitivity of the lignin peroxidase production to agitation and shear stress, the utilization of immobilized *Phanerochaete chrysosporium* has been studied in different reactor configurations (Pak 1988; Lewandowski 1988; Haq 1989). Haq (1989) studied the degradation in three different reactor configurations, namely a batch reactor, a chemostatic reactor with the fungus immobilized on a silica based porous biocatalyst, and a packed bed reactor utilizing balsa wood chips as a packing. He concluded that the fungus is not active when grown in suspension in a well agitated fermenter. However, the degradative activity may be increased by allowing the fungus to grow on and into porous support particles, either suspended in an agitated fermenter or fixed in a packed column. Similar results were obtained by Pak (1988) who immobilized the fungus on porous silica beads in a packed column.

2.1.1.6 Effect of Veratryl Alcohol

Harvey et al. (1986) reported that the role of veratryl alcohol in ligninase degradation was as an enzyme mediator. Veratryl alcohol was oxidized to a radical cation which was not rapidly degraded. Therefore, it could act as a one-electron oxidant, and during the electron transfer process, it was regenerated. It was suggested that the lignin degrading enzyme functioned not as an oxygenase but a peroxidase, and that the oxidation reaction was brought about by the initial single electron transfer between the aromatic ring and an active site in the enzyme.

2.1.1.7 Effect of Trace Nutrients

Kirk et al. (1986) described a method for increasing the production of ligninase by the fungus involving the addition of veratryl alcohol and excess trace metals to stationary flask cultures. The control ligninase activity increased approximately five folds as a result of these additions. They carried out different

sets of experiments using defined trace metals in the media and concluded that Cu^{+2} and Mn^{+2} ions are the most important trace metals for ligninase activity. They showed that veratryl alcohol and trace metals have additive effects, indicating that they have different sites of action.

2.1.1.8 Effect of Temperature

The white rot fungus grows over a wide range of temperatures. Growth has been assessed from 10-39°C (Glaser, 1988). No growth is observed at 10°C, whereas growth significantly increases with temperature from 15 to 40°C. Kirk et al. (1978) suggested an optimum temperature of 39°C for growth with agitated cultures.

2.2 Degradation in Soil and Sand

The general success of the liquid phase biodegradation studies using this fungus indicates that this microorganism can be an appropriate candidate for the treatment of contaminated soils and sand. However, there are very few published articles available regarding the activity of this fungus in soil.

Glaser et al. (1989) studied the effect of different parameters on the degradation of xenobiotics in soil. Three well characterized soils were used in their studies. The effect of different soil type, temperature, and water potential on the growth of the fungus in soil was studied in a series of factorial experiments.

Lamar et al. (1989) studied the fate of pentachlorophenol (PCP) in soils inoculated with *Phanerochaete chrysosporium*. They measured the mineralization and volatilization of PCP, as well as its transformation products, over a two month period using soil inoculated with fungus or left uninoculated. They

observed a dramatic decrease (about 98%) in the extractable PCP concentration in inoculated soils compared to that in uninoculated soils. They suggested that *Phanerochaete chrysosporium* removes PCP primarily by converting it to non-volatile products.

Bumpus et al. (1990) studied degradation of DDT and selected PAHs in creosote contaminated soil. They obtained about 80% and 11% degradation of PAHs and DDT, respectively.

2.2.1 PROCESS PARAMETERS AFFECTING THE BIODEGRADATION PROCESS IN SOIL AND SAND

In order to achieve the optimum ligninase activity in soil or sand, it is important to optimize the factors contributing to growth of the fungus. The most important of these parameters are as follows:

2.2.1.1 Effect of Carbon Source

Since *Phanerochaete chrysosporium* can not utilize xenobiotics as a primary carbon source, it is important to provide glucose or another suitable carbon source to the carbon deficient soils. This is especially important in the case of sand which does not typically contain any carbon source.

Lamar et al. (1989) studied the degradation of pentachlorophenol in Marshan sandy loam (fine-silty, mixed, mesic, mollic-Hapludalf), Xurich sandy loam (fine-silty, over sandy, mixed, mesic, Typic-Haplaquoll) and Batavia silty clay loam (fine-silty, mixed, mesic, Typic-Hapludalf). They obtained the highest rate of

degradation in Marsham soil. They suggested that the difference among these soils is principally the quantity of soil nutrients available for fungal growth, particularly carbon and nitrogen.

Bumpus et al. (1990) studied the degradation of DDT in soil using *Phanerochaete chrysosporium*. They used a variety of very economical and readily available substrates such as corn-cobs, newspaper, and wheat straw as carbon sources. Of the growth substrates tested, ground corn-cob appeared to be the most effective in supporting fungal growth. Studies revealed that the amount of DDT mineralization increased linearly with increasing ratio of corn cobs to DDT-contaminated soil, up to approximately 4:1.

2.2.1.2 Effect of Moisture

In order to support the growth of the fungus, it is important to have moisture in the soil. Bumpus et al. (1990) studied the effect of moisture content of the soil on the rate of degradation of DDT. They observed that the rate of mineralization of DDT is low at very high or low moisture content. They suggested a moisture content of 40% to be optimal. Similar results were obtained by Lamer et al. (1990) who measured the moisture content in terms of the water potential of the soil.

2.2.1.3 Effect of pH

Using ^{14}C-DDT as a model compound, Bumpus et al. (1990) carried out several experiments in soil and suggested that the optimum rate of degradation is obtained over a broad pH range of 3.5 to 6.0. This is contradictory to literature data for lignin degradation, which indicate an optimum pH of 4.5. However, no other results are available in the literature.

2.2.1.4 Effect of Temperature

Kirk et al. (1978) suggested an optimum temperature for the growth of fungus in agitated cultures as 39^{0}C. However, Glaser (1989) found that the growth of the fungus in soil was unaffected between 25^{0}C and 35^{0}C, but significantly decreased at 39^{0}C. This is another discrepancy between the optimum conditions in soil and agitated cultures.

Chapter 3

Materials and Methods

3.1 Organism and Inoculum

Phanerochaete chrysosporium BKM-1767 (ATCC 24725) was obtained from the American Type Culture Collection (ATCC) and from the Department of Wood and Paper Science, North Carolina State University. The ATCC culture was maintained on potato dextrose agar media. Potato dextrose agar medium was prepared by boiling 300 gram of diced potatoes in about 500 gm distilled and deionized water until thoroughly cooked and filtered through cheese cloth. The volume of filtrate was brought to one liter with distilled water. 15 gram of agar and 20 gram of glucose were added to it prior to autoclaving.

The culture from North Carolina State University was maintained on yeast malt extract agar medium. Yeast malt extract media was prepared by dissolving 0.3 gram of yeast extract, 0.3 gram of malt extract, 0.5 gram of peptone, 2 gram of agar and 1 gram of glucose in 100 gm of warm distilled and deionized water prior to autoclaving. After autoclaving the medium was cooled to 50-60^{0}C.

3.2 Degradation in Wastewater

3.2.1 Culture Media

All media and solutions were prepared using distilled and deionized water.

Growth Medium

The growth medium was used to grow *Phanerochaete chrysosporium* for wastewater treatment experiments had the following composition:

KH_2PO_4	2.00 g
$MgSO_4$	0.50 g
$CaCl_2$	0.10 g
NH_4Cl	0.12 g
Glucose	10.00 g
Thiamine	1.00 mg
Mineral Salt Solution	5.00 ml
Water	1.00 lit

The final pH of the medium was 4.2-4.5.

Induction Medium

In the packed bed reactor experiments, the growth medium was replaced with a induction medium after the fungal growth period was completed. The induction medium had low concentrations of nitrogen and carbon in order to stimulate enzyme production. The composition of the induction medium was:

KH_2PO_4	2.00 g
$MgSO_4$	0.50 g
$CaCl_2$	0.10 g
NH_4Cl	12.00 mg
Glucose	2.00 g
Thiamine	1.00 mg
Mineral Salt solution	5.00 ml
Water	1.00 liter

The final pH of the induction medium was 4.2-4.5.

Mineral Salt Solution

A mineral salt solution was added to the growth medium to provide the fungus with those trace elements required for growth as well as production of the ligninolytic enzyme system. The composition of the mineral salt solution was as follows:

KH_2PO_4	0.20 g
$MgSO_4.7H_2O$	3.00 g
$CaCl_2$	0.10 g
$MnSO_4.2H_2O$	0.50 g
NaCl	1.00 g
$FeSO_4.7H_2O$	0.10 g
$CoSO_4$	0.10 g
$ZnSO_4$	0.10 g
$CuSO_4.5H_2O$	10.00 mg
$AlK(SO_4)_224H_2O$	10.00 mg

H_3BO_3	10.00 mg
$NaMoO_4$	10.00 mg
Nitriotriacetate	1.50 g
Water	1.00 liter

5 ml of mineral salt solution was added to 1 liter of growth medium.

3.2.2 Lindane Additions

Since solubility of lindane in water is only 10 ppm at room temperature, it is very difficult to prepare lindane solutions of precise composition in water. Therefore, stock solutions were prepared in acetone. 3 g of lindane were dissolved into 100 ml of acetone to give a 30,000 ppm solution. In the packed bed reactor experiments, 3.3 ml of this lindane solution were mixed with 10 liters of induction medium. The final concentration was about 9.8-9.9 ppm. For the shaker flask studies, a 10,000 ppm solution was prepared in the same way, and 0.1 ml of this solution were added to 100 ml growth medium so as to give a 10 ppm solution in water.

3.2.3 Shaker Flask Studies

As a first experiment in shaker flasks, 0.1 ml of the 10,000 ppm lindane solution in acetone were added in 100 ml growth medium at the time of inoculation. Conidia from an agar plate were used as inoculum. It was found that the white rot fungus grows well in the presence of lindane, suggesting that there is no toxic effect on fungal growth up to a concentration of 10 ppm.

In the second set of shaker flask studies, the fungus was allowed to grow in 100 ml growth medium for five days. On the sixth day, 0.1 ml of 10,000 ppm lindane solution were added to each flask. In order to find the losses due to

volatilization, control cultures consisting of growth medium only were also run. Samples from each flask were collected at regular intervals. The samples were analyzed for lindane concentration.

3.2.4 Experiments in the Packed Bed Reactor System

A schematic diagram for the packed bed reactor is shown in Figure 1. A plastic (Lucite) pipe, 4 inch ID and 26 inch in length, closed at both ends, was used as a packed bed reactor. Saw dust was used as the packing in these experiments. The reactor working volume was 4.5 liters. The void volume was found to be 1.65-1.7 liters for different experiments, i.e. the void fraction in the reactor was 0.37.

It is very important to know the residence time distribution of the fluid flowing through the reactor in order to develop a suitable model for it. This information was determined directly by a widely used method of inquiry, the stimulus-response experiment (Levenspiel, 1972, page 254). In order to characterize the extent of non ideal flow, a residence time distribution (RTD) study was conducted in the reactor. An aqueous copper sulfate solution having a concentration of C_0 (0.09 gm/lit) was used as a tracer in the experiment. Polypropylene plastic chips were used as a packing material instead of the saw dust used in the experiments with the fungus, since copper sulfate is not adsorbed on the plastic. The void fraction was found to be the same as with saw dust. The flow rate at the inlet was kept to 2 ml/min. With no tracer initially present in the column, a step input of tracer of concentration C_0 was imposed on the fluid entering the vessel by replacing the flask filled with copper sulfate solution (tracer) instead of water. A time record of the tracer concentration at different sample ports up the column was determined as C/C_0, using a Varian DMS 200 UV-visible spectrophotometer. The

dispersion number (D/uL) characterizing the type of flow was determined from the data, where D is dispersion coefficient and u is a superficial velocity of the fluid.

In the packed bed experiments, saw dust was used as a packing material. The temperature of the system was held constant by circulating water in the reactor jacket. Conidia from the agar plate was used as inoculum and the fungus was allowed to grow on the surface of the packing material for five days by circulating 5.0 liters of growth medium in a closed loop. On the 6th day, the reactor was constantly fed with induction medium containing 9.8-9.9 ppm lindane introduced from the bottom of the reactor. The overflow from the reactor was separately collected. Samples from different ports were analyzed for lindane, glucose and nitrogen concentration. The experiment was carried at room temperature (25^{0}C).

Since hydrogen peroxide was the oxygen source used in the packed bed reactor (and the vapour pressure of lindane at room temperature is about 10^{-6} mm Hg), there were no significant volatilization losses.

3.2.5 Methods of Analysis

3.2.5.1 Lindane Analysis

A gas chromatograph (HP 5890) equipped with Electron Capture Detector was used to determine the concentration of lindane in the samples. A 10 ml sample was taken from the different sample ports of the reactor and centrifuged for 10 minutes at 5500 rpm. One ml of this sample was placed in a 20 ml test tube and 1 ml of n-heptane was added into it, in order to extract the lindane. 0.3 gram of NaCl and 2 drops of 5M HCl were added to the sample in order to increase

polarity. The sample and the n-heptane were mixed using a vortex mixer. After mixing, the n-heptane phase was separated and transferred to another 20 ml tube. This procedure was repeated three times for each sample (i.e. extraction of lindane was carried out in three stages). All these n-heptane fractions were collected together and then diluted with n-heptane so as to give a concentration of about 0.003 ppm lindane in n-heptane. 0.1 g of anhydrous $CaCl_2$ were added in order to remove any moisture present in the sample. One micro-litre of this sample was injected into the gas chromatograph. The temperatures of the oven, injector and detector were kept at 170°C, 200°C and 300°C, respectively. Nitrogen was used as carrier gas. A Hewlett Packard 3396 electronic integrator was used to determine the peak area in the chromatogram.

3.2.5.2 Glucose Analysis

The o-toluidene method was used to determine the glucose concentration in the samples. At 100°C, o-toluidene reacts with glucose in the presence of an acid to form a blue green color complex. The intensity of the color is proportional to the glucose concentration (Sigma Method 635-6).

0.5 ml of EDTA solution were added to 10 ml of a sample collected from the fermenter, and centrifuged for 10 minutes at 5500 rpm. 0.1 ml sample, 0.1 ml of standard glucose (Sigma-635-100), and 0.1 ml of distilled water were placed into three separate test tubes. 5 ml of o-toluidene solution (Sigma-635-6) were added to each test tube. The tubes were placed in a boiling water bath for exactly 10 minutes, after which they were quenched in cold water for three minutes. The contents of the tubes were transferred to cuvets and their absorbance was measured at 635 nm using a Varian DMS 200 UV-Visible spectrophotometer, taking the blank as a reference.

3.2.5.3 Nitrogen Analysis

Since NH_4Cl was used as a nitrogen source in the growth medium as well as in the induction medium, an Orion ammonia electrode (model 95-12) was used to measure the nitrogen concentration of each sample during fermentation. An Orion model 701-A meter was used to measure the mV response of the electrode immersed in the sample. The mV reading was transformed into the corresponding nitrogen concentration using a calibration curve obtained as described below.

Nitrogen standard solutions were prepared by dissolving ammonium chloride in one liter of water. 1000, 100, 10 and 1 ppm nitrogen standards were prepared. The ammonia electrode was placed in 50 ml of 10 ppm nitrogen standard in a 100 ml beaker. 0.5 ml of a pH adjusting solution (Orion no. 951211) was also added. The reading of the 10 ppm nitrogen standard was arbitrarily set to zero. This calibration procedure was then carried out for other standards. The resulting calibration curve [electrode potential in mV vs. log(ppm N_2)] was linear in the range 0.1 to 1000 ppm.

For each measurement, the electrode was first placed in a 10 ppm nitrogen standard containing 0.5 ml of pH adjusting solution. The corresponding mV reading on the meter was set to zero. The electrode was then washed with water and placed in 50 ml of unknown solution containing 0.5 ml of pH adjusting solution. The mV reading on the meter was noted. The corresponding nitrogen concentration was obtained from the calibration curve.

3.2.5.4 Protein Analysis

Protein analysis was carried out using BCA Protein Assay Reagent. This reagent is a highly sensitive reagent for the spectrophotometric determination of protein concentration (Pierce method 23320, 23225). Protein reacts with Cu^{+2} yielding Cu^{+1} which reacts with BCA reagent to make BCA-Cu^{+1} complex. This complex exhibits a strong absorbance at 562 nm. This allows the spectrophotometric quantification of protein in aqueous solutions. A set of protein standards of known concentration were prepared using bovine serum albumin, and a standard curve of absorbance against protein concentration was generated.

10 ml of sample were taken in a test tube and centrifuged at 5000 rpm for about 10 minutes in order to remove wood dust from the sample. 0.1 ml of supernatent were taken and 2 ml of working reagent were added to it, and it was then incubated for 30 minutes at 37^{0}C. The increase in absorbance was measured at 562 nm using Varian DMS 200 UV-visible spectrophotometer. The protein concentration was found using the standard curve.

3.3 Experiments in Sand

3.3.1 Sand Preparation

The sand was obtained from the construction site of the Information Technology Center at the New Jersey Institute of Technology, Newark, NJ. The sand was washed several times with water in order to remove inorganic and organic matter present in it. It was then dried at 100^{0}C for 24 hours. The dry sand was sieved to remove the course aggregates. The sand with an average particle size of 425 ± 25 micron was used in all the experiments. The sand was sterilized by autoclaving at 121^{0}C for 25 minutes.

A 10 ppm Lindane solution in acetone was prepared as described previously. 20 gram of the sterilized sand were taken into 50 ml polyethylene tubes and 20 ml of the acetone-lindane solution were added to it. The sand-acetone slurry was mixed with a vortex mixer for about 10 minutes. The acetone carrier was allowed to evaporate for 96 hours, resulting in an artificially contaminated sand.

To assess the ability of *Phanerochaete chrysosporium* to degrade lindane, 20 g of sand having 10 mg/kg lindane were placed into 250 ml sterile Erlenmeyer flasks. A control experiment was also run in order to find the losses due to volatilization or possible contamination by other species.

3.3.2 Inoculum Preparation

Two different methods were used to inoculate the sand. In the first method, *Phanerochaete chrysosporium* was first grown on malt agar slants for one week, and then stored at 4^0C. A soil inoculum consisted of Balsa wood chips (0.5 cm dia, 1 cm long) thoroughly grown with *Phanerochaete chrysosporium.* The chips had been previously sterilized by autoclaving at 121^0C for one hour. The wood chips were saturated with water prior to autoclaving by immersing them in distilled and deionized water for 24 hours. The chips were inoculated by incubating them with bits of malt agar from *Phanerochaete chrysosporium* slants. Inoculated chips were incubated at 39^0C for one week. Five chips were added to each flask.

In the second method, 100 ml of growth medium were taken, and conidia from the agar plates were used to inoculate it. The fungus was allowed to grow in the growth medium for seven days on the shaker. The contents of these flasks were homogenized in the mixer, and then used to inoculate the sand by adding 1 ml growth medium containing fungal spores in each flask containing 20 gm sand.

3.3.3 Experimental Set-up

Prior to the beginning of the experiments all the flasks were sterilized at 121°C for 25 minutes. The sand contaminated with lindane was added aseptically to each flask, and these flasks were inoculated either with growth medium containing fungal spores or with the wood chips thoroughly grown with fungus as described previously. These flasks were maintained at 25°C.

In order to find the losses due to volatilization or contamination, control experiments were also carried out. Control cultures consisted of uninoculated sand. Different parameters were studied to find the optimum rate of degradation. It was found that the presence of carbon and moisture content are crucial factors in achieving a maximum degradation rate.

A series of experiments were run in order to determine the optimum level of carbon source and moisture content. In the first set of experiments the glucose concentration was kept at 10 gm/kg of sand, and in the second set it was kept at 20 gm/kg of sand. For each set of experiments, the moisture content was 20%, 40% and 60%. One inoculated and one control culture were harvested each week for 5 weeks. The control experiments included 10 gm glucose/kg of sand, and 40% moisture.

3.3.4 Lindane Analysis

In order to confirm the amount of lindane at day 0, the analysis was performed on one culture in each set of experiment. Before sampling, the sand in each flask was thoroughly mixed. These sand samples were extracted twice with 10 ml of n-heptane. After extraction, 1 gram of anhydrous calcium chloride was added

to the n-heptane extract to remove traces of water. Recovery experiments in flasks containing a known amount of lindane indicated that the extraction efficiency was 97.8%.

A gas chromatograph (HP5890) was used to determine the concentration of lindane in the samples. 1 microlitre of sample was injected into 6 ft x 530 micrometer chromatographic column. The temperature of oven, injector and detector were kept at 170^{0}C, 200^{0}C and 300^{0}C respectively. A Hewlett-Packard 3396 integrator was used to determine the peak area.

Chapter 4

Results and Discussion

4.1 Biodegradation of Lindane in Wastewater

4.1.1.1 Shaker Flask Cultures

Shaker cultures were used for:

a) producing enough biomass to be used as inoculum for packed bed reactor;

b) determining the ability of *Phanerochaete chrysosporium* to degrade lindane.

In the shaker studies, the fungus grew in the form of pellets. *Phanerochaete chrysosporium* was exposed to lindane, five days after the initial inoculum. Table 1 and Figure 2 shows the results obtained for the degradation of lindane. In order to account for losses due to volatilization or adsorption of lindane on the wall of flask, control experiments having no fungus were also run. The control experiments resulted in a small lindane loss (about 20%) compared to the inoculated flasks.

4.1.1.2 Spike Experiments

In order to confirm the shaker flask study data and rule out the possibility that adsorption on the biomass could be responsible for the results obtained, a spiking study was also performed in which the concentration of lindane was readjusted back up to its initial value once it had dropped below 5 ppm. Table 2 and Figure 3 represents the data obtained from this study. This study also showed the ability of the fungus to degrade lindane. However, it was found that the activity of the fungus decreases with time. The biomass became brownish in color.

4.1.2 Biodegradation of Lindane in Packed Bed Reactor

Immobilization of *Phanerochaete chrysosporium* is very crucial for effective induction of the appropriate degradative enzyme system. Additional advantages of cell immobilization are as follows:

a) it provides a high cell concentration, and therefore higher reaction rates, per unit volume of reactor;

b) it eliminates the need for fungus separation and recycle;

c) it protects the fungus against high shear-stress.

Therefore, to induce the appropriate degradative enzyme system, the utilization of immobilized *Phanerochaete chrysosporium* was studied in a packed bed reactor. Table 3 and Figure 4 show the results for mineralization of lindane in the packed bed reactor. Table 4 and Figure 5 represent the change in protein and glucose concentration along the length of packed bed reactor. Table 5 and Figure 6 shows the change in pH along the reactor. It was found that pH decreases along the length of reactor; suggesting that the fungus is active. The concentration of lindane, protein and glucose at different sample ports was found to be different, suggesting that the reactor does not behave like an ideal mixed flow reactor, but rather like a plug flow reactor.

In order to study the extent of deviation from the ideal plug flow reactor, a residence time distribution study was carried out. A copper sulfate solution was injected continuously at the bottom of the reactor as described in the Materials and Methods section. The absorbance was found at the second sample port instead of the first sample port in order to avoid any errors due to mixing effects at the entrance. The actual residence time was found to be 4.88 hrs, and the dispersion number (D/ul), a measure of the extent of axial dispersion, was found to

be 0.012 (as shown in Appendix 1), suggesting that the reactor behaves very much like an ideal plug flow reactor (Levenspiel, 1972, page 276).

Assuming first-order kinetics for the degradation of lindane in the reactor, the following mass balance can be written for a differential segment of the reactor:

$$Q\,C_c - Q\,(C_c - dC_c) = -K\,C_c\,dV$$

$$Q\,dC_c = -K\,C_c\,dV$$

$$Q\,dC_c/dV = -K\,C_c$$

$$(Q/A)\,dC_c/dX = -K\,C_c$$

After integration the above expression becomes,

$$\ln(C_c/C_{co}) = -K\,(A/Q)\,X$$

Where,

V= Volume of the reactor (ml)

Q= Volumetric flow rate (ml/min)

C_o= Lindane concentration in feed (mg/ml)

C_c= Lindane concentration at a distance X (mg/ml)

K= First-order rate constant (min^{-1})

The first-order rate constant was found to be 0.0066 min^{-1}. The correlation can satisfactorily predict the lindane degradation rate in the packed bed reactor with

an error of about 20%. The regressed equation is also shown in Figure 4 as a solid line.

4.2 Biodegradation of Lindane in Sand

In order to determine the rate of degradation in sand, two different types of experiment were carried out. In the first type of experiments, the sand was inoculated with the fungus grown in growth media for five days (Direct Inoculum). In the second type of experiments, the sand was inoculated with fungus grown on wood chips as described previously. It is thought that the essential nutrients required for the growth of the fungus come from the growth media added along with fungus during direct inoculum, while for the wood inoculum the fungus gets all nutrients from the wood chips.

4.2.1 Direct Inoculum

In the first set of experiments the initial glucose concentration was kept at 10 gm/kg of sand, and the moisture content was adjusted to 20%, 40% and 60% w/w at the beginning of the experiment. (Glucose concentration in the water was 5% w/w, 2.5% w/w and 1.66% w/w respectively.) Tables 6,7 and 8 and Figure 7 show the results obtained for these set of experiments.

Assuming a first-order mechanism for the degradation of lindane in the reactor, a mass balance can be written as follows:

$$dC_c/dt = -K C_c$$

Integrating,

$$-\ln(C_c/C_{co}) = k t$$

Where,

C_c = Lindane concentration at time t (mg/kg of sand)

C_{co} = Lindane concentration at time t=0 (mg/kg of sand)

t= Time (day)

k= First order rate constant (day^{-1})

The regressed equation is shown in Figure 7 as solid lines. The rate constants obtained after the regression of the data are 0.072 day^{-1}, 0.095 day^{-1} and 0.035 day^{-1} for 20%, 40% and 60% w/w moisture content respectively. It was found that the 40% w/w moisture content resulted in the highest degradation rate.

In the second set of experiments, the initial glucose concentration was increased to 20 gm/kg of sand and the initial moisture content adjusted to 40% and 60% w/w, respectively. (Glucose concentration in water was 5% w/w and 3.33% w/w respectively.) Tables 9 and 10 and Figure 8 represent the data obtained in these experiments. The first order degradation constant was found to be 0.109 day^{-1} and 0.060 day^{-1}, for 40% and 60% moisture content respectively. It was found that the degradation constants obtained from this experiment were higher than those obtained with 10 gm glucose/kg of sand, suggesting that the glucose is essential for the activity of fungus.

In order to study the losses due to contamination or adsorption of lindane on the wall of the flask, a set of control experiment was performed. The glucose concentration was kept at 10 gm/kg of sand. The loss of lindane in this control experiment was found to be negligible, as shown in Table 11.

4.2.2 Inoculum- Fungus Grown on Wood Chips

In order to find a better method of inoculation, wood chips thoroughly grown with fungus were used as the inoculum.

In the first set of experiments no glucose was added to the sand and initial moisture content was adjusted to 40% w/w. Tables 12 and 13 and Figure 9 show the results obtained from these experiments. Control experiment showed no losses of lindane, indicating that biodegradation was indeed taking place and accounted for the entire loss of lindane. A lag phase of about 14 days was found in this experiment before any significant degradation could be observed.

In the second set of experiments, the initial glucose concentration was kept 10 gm/kg of sand and initial moisture content was adjusted to 40% and 60% w/w respectively. Table 14 and 15 and Figure 10 represents the data obtained in these experiments. The first order degradation constants obtained after regression are 0.088 day^{-1} and 0.054 day^{-1}, for 40% and 60% moisture respectively, suggesting that this technique is not as effective as direct inoculation with conidia.

Chapter 5

Conclusions

5.1 Biodegradation in Water

1) *Phanerochaete chrysosporium* is able to degrade lindane (hexachlorocyclohexane) in water up to a concentration of 10 ppm (solubility limit of lindane= 10 ppm).

2) Packed bed reactor used in this study with a flow rate of 2 ml/min and H_2O_2 as the oxygen source behaves like an ideal plug flow reactor.

3) Saw dust can be used as a packing material, instead of porous silica based beads or wood chips used by earlier invesigators.

5.2 Biodegradation in Sand

1) *Phanerochaete chrysosporium* is able to degrade lindane (hexachlorocyclohexane) up to a concentration of 10 mg/kg in sand.

2) The moisture content of the sand appears to have an important effect on the rate of biodegradation, with an optimum of about 40 wt%.

3) Method of growing *Phanerochaete chrysosporium* on wood chips, which is then used subsequently for sand inoculation, results in lower rates of degradation than when conidia are added directly to the sand.

Chapter 6

Future Work

6.1 Biodegradation in Wastewater

1) The enzyme production kinetics should be studied in detail.

2) The performance of the packed-bed reactor configuration should be tested using real waste.

3) Other recalcitrant compounds of industrial interest should be tested.

6.2 Biodegradation in Sand

1) Use of other carbon sources which are cheap and readily available (such as corn-cob, starch etc.) should be investigated.

2) Suitable analytical methods for determination of the amount of biomass should be developed.

3) The fractional factorial type of study should be carried out in order to find the combined effect of moisture content and supplemental carbon source.

4) The effect of temperature on the rate of degradation in sand should be investigated.

5) *Phanerochaete chrysosporium* should be tested against other recalcitrant compounds of industrial relevance.

6) *Phanerochaete chrysosporium* should be tested against actual contaminated sand and soil.

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Table 1
Biodegradation of Lindane in Wastewater
Shaker Flask Studies

Time	Lindane			
hrs.	Inoculated			Control
	Expt 1	Expt 2	Expt 3	
0	9.87	8.46	9.93	8.02
48	8.9	7.55	9.21	7.55
96	6.15	6.21	5.86	7.26
192	3.53	4.53	2.72	6.85
240	3.41	3.34	2.56	6.23

Table 2
Biodegradation of Lindane in Wastewater
Spike Studies in Shaker Flasks

Time hrs.	Lindane	ppm
	Expt 1	Expt 2
0	9.97	9.91
72	6.25	7.09
120	4.07	4.6
168	3.1	4.3
192	3.03	4.2
192 (Spike)	10.01	8.9
264	6.92	4.88
288	6.2	4.78
312	5.9	4.75
312 (Spike)	9.3	9.05
384	7.84	7.84
408	7.22	7.03
432	7.01	6.85

Table 3
Biodegradation of Lindane in Packed Bed Reactor
Flow Rate 2 ml/min

Sample Port No.	Lindane ppm
Inlet	9.97
1	9.5
2	5.88
3	4.06
4	3.85

Table 4
Change in Protein and Glucose Concentration in Packed Bed Reactor
Flow Rate 2 ml/min

Sample Port No.	Protein Conc. ug/ml	Glucose Conc. gm/lit
Inlet	0.0	1.95
1	35	1.71
2	51	1.04
3	65	0.83
4	80	0.49

Table 5
Change in pH in Packed Bed Reactor

Sample Port No.	pH
1	5.3
2	5.22
3	5.08
4	4.94

Table 6
Biodegradation of Lindane in Sand
Glucose 10 gm/kg of sand; 20% Moisture
Direct Inoculum

Time days	Lindane mg/kg of sand
0	10.0
10	5.52
17	2.47
23	1.84
32	1.52
37	0.00

Table 7
Biodegradation of Lindane in sand
Glucose 10 gm/kg of sand; 40% Moisture
Direct Inoculum

Time days	Lindane mg/kg of sand
0	10.0
10	3.58
17	0.57
23	1.37
32	1.13
37	0.23

Table 8
Biodegradation of Lindane in Sand
Glucose 10 gm/kg of Sand; 60% Moisture
Direct Inoculum

Time days	Lindane mg/kg of sand
0	10.0
10	8.4
17	5.4
23	4.56
32	3.99
37	3.81

Table 9
Biodegradation of Lindane in Sand
Glucose 20 gm/kg of Sand; 40% Moisture
Direct Inoculum

Time days	Lindane mg/kg of sand
0	10.0
10	4.36
17	0.69
23	0.19
32	0.20
37	2.10

Table 10
Biodegradation of Lindane in Sand
Glucose 20 gm/kg of Sand 60% Moisture
Direct Inoculum

Time days	Lindane mg/kg of sand
0	10.0
10	6.74
17	3.61
23	3.02
32	1.99
37	1.31

Table 11
Biodegradation of Lindane in Sand
Control Experiment
Glucose 10 gm/kg of sand; 40% Moisture

Time days	Lindane mg/kg of sand
0	10.0
7	9.87
14	9.64
21	9.73
28	9.53
35	9.02

Table 12
Biodegradation of Lindane in Sand
No Carbon Source; 40% Moisture
Inoculum with fungus grown on wood chips

Time days	Lindane mg/kg of sand
0	10.0
7	10.0
14	10.2
21	5.1
28	5.2

Table 13
Biodegradation of Lindane in Sand
No Carbon Source Control Experiment
Sterile wood chips without fungus

Time days	Lindane mg/kg of sand
0	10.0
7	9.8
14	9.7
21	9.5
28	9.3

Table 14
Biodegradation of Lindane in Sand
Glucose 10 gm/kg of Sand; 40% Moisture
Inoculum with fungus grown on wood chips

Time days	Lindane mg/kg of sand
0	10.0
7	4.1
14	2.64
21	2.43
28	0.87
35	1.25

Table 15
Biodegradation of Lindane in Sand
Glucose 10 gm/kg of Sand; 60% Moisture
Inoculum with fungus grown on wood chips

Time days	Lindane mg/kg of sand
0	10.0
7	7.76
14	4.47
21	2.91
28	0.87
35	0.04

Table 16
First order rate Constant for the Biodegradation of Lindane in Sand

Direct Inoculum		
Glucose Conc.	Moisture Content	Rate Constant
		day-1
10	20	0.072
10	40	0.095
10	60	0.035
20	40	0.105
20	60	0.053
Inoculum with fungus grown on wood chips		
10	40	0.074
10	60	0.056

Schematic Diagram of Experimental Set-up for Packed-Bed Reactor

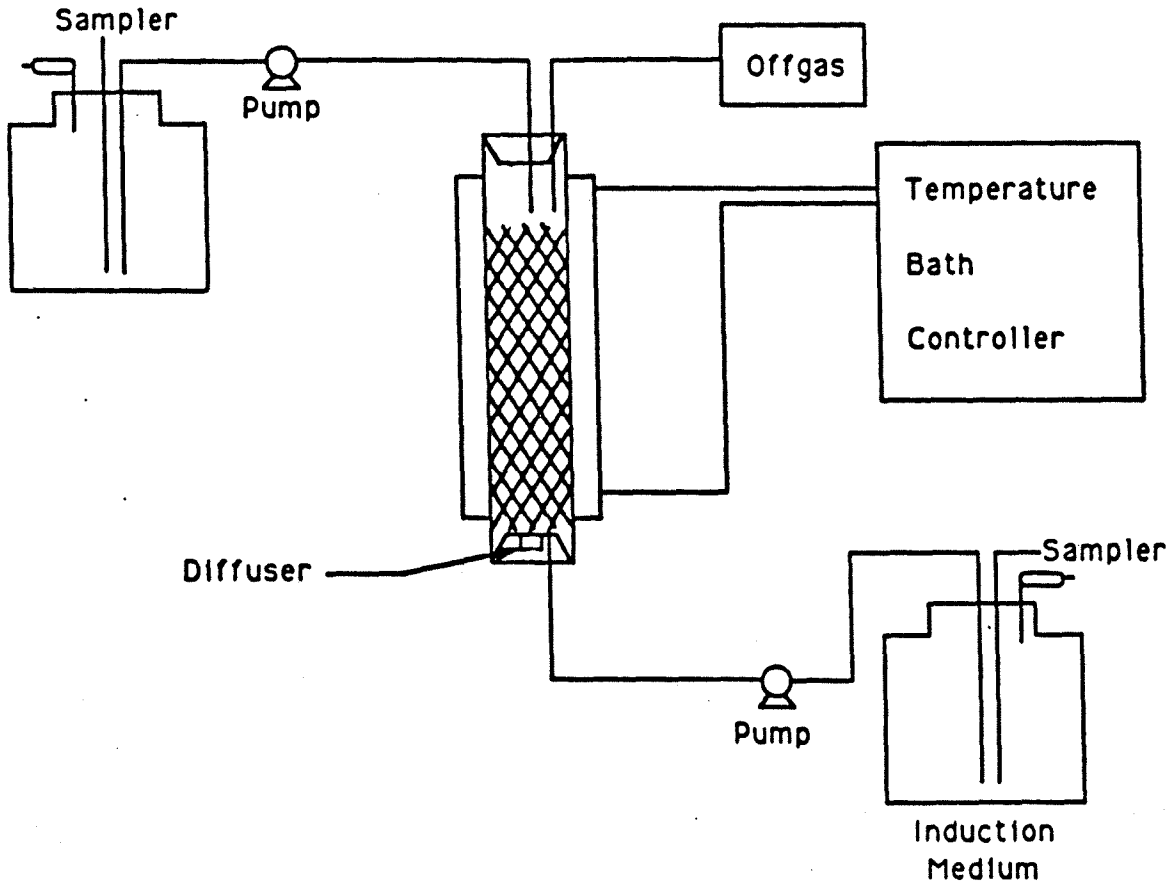

Figure 1

Biodegradation of Lindane in Water
Shaker Flask Studies

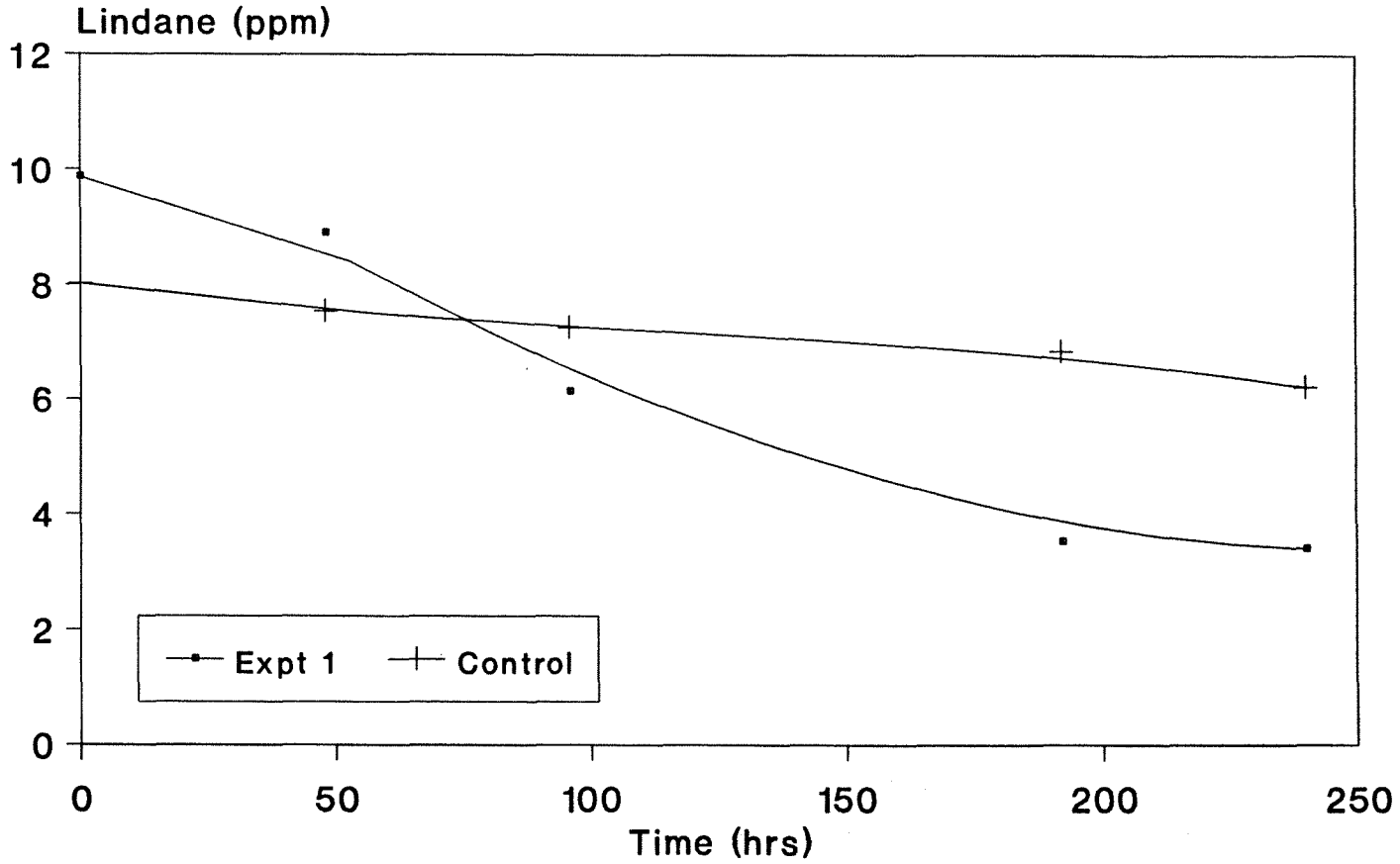

Figure 2

Biodegradation of Lindane in Water
Spike Studies in Shaker Flasks

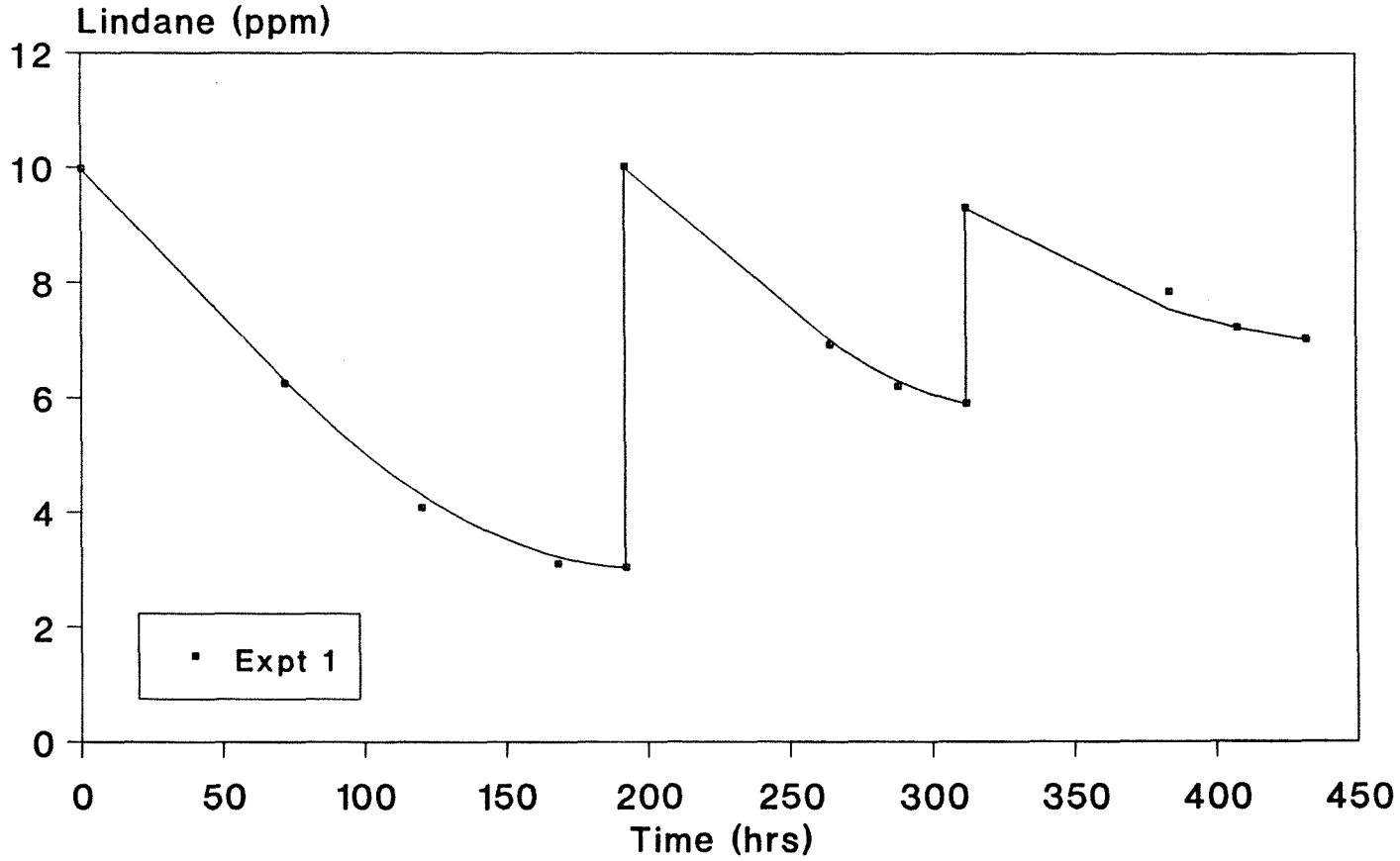

Figure 3

Biodegradation in Packed Bed Reactor

Flow Rate = 2 ml/min

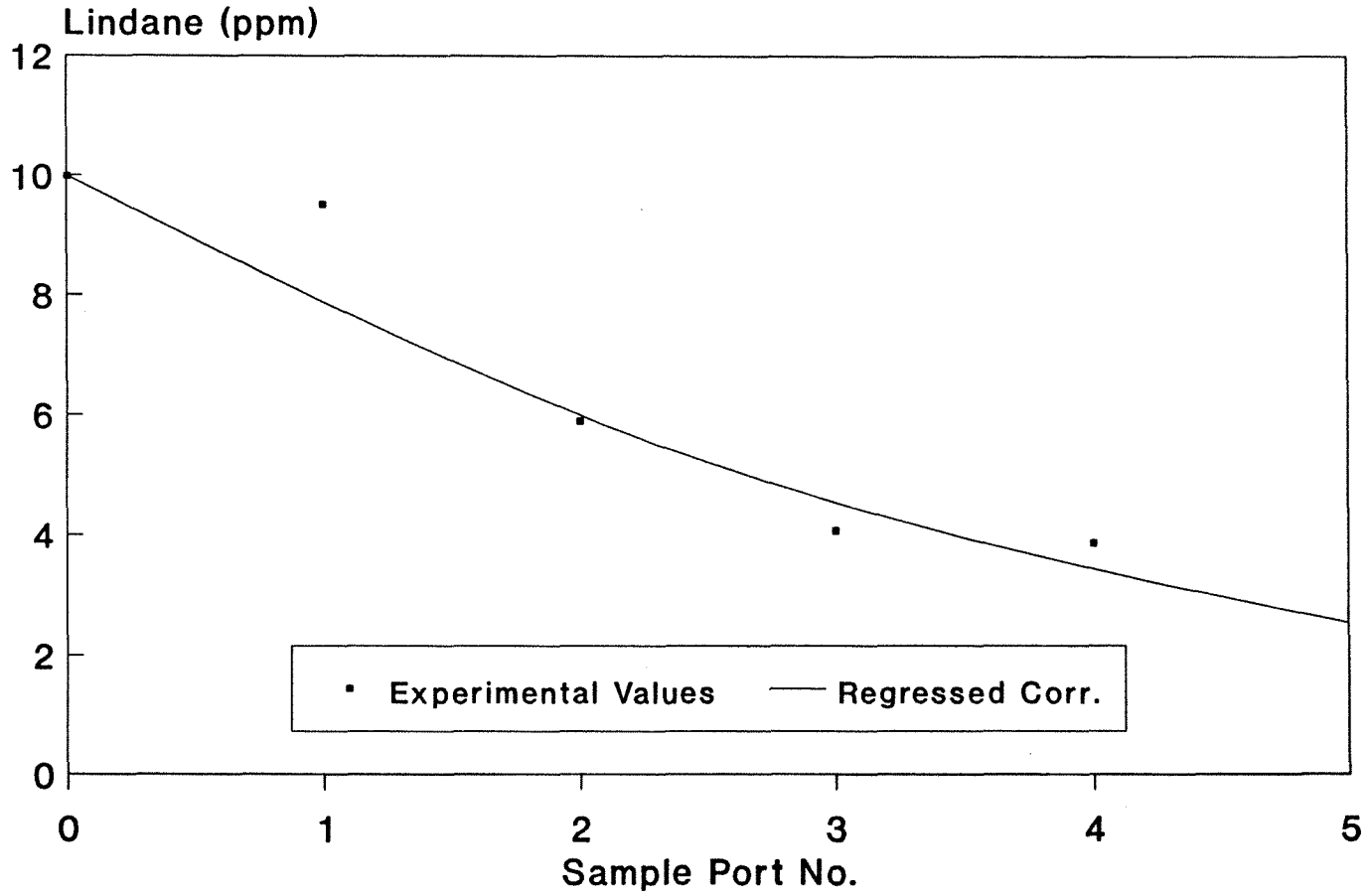

Figure 4

Change in Protein and Glucose Conc.

Flow Rate = 2 ml/min

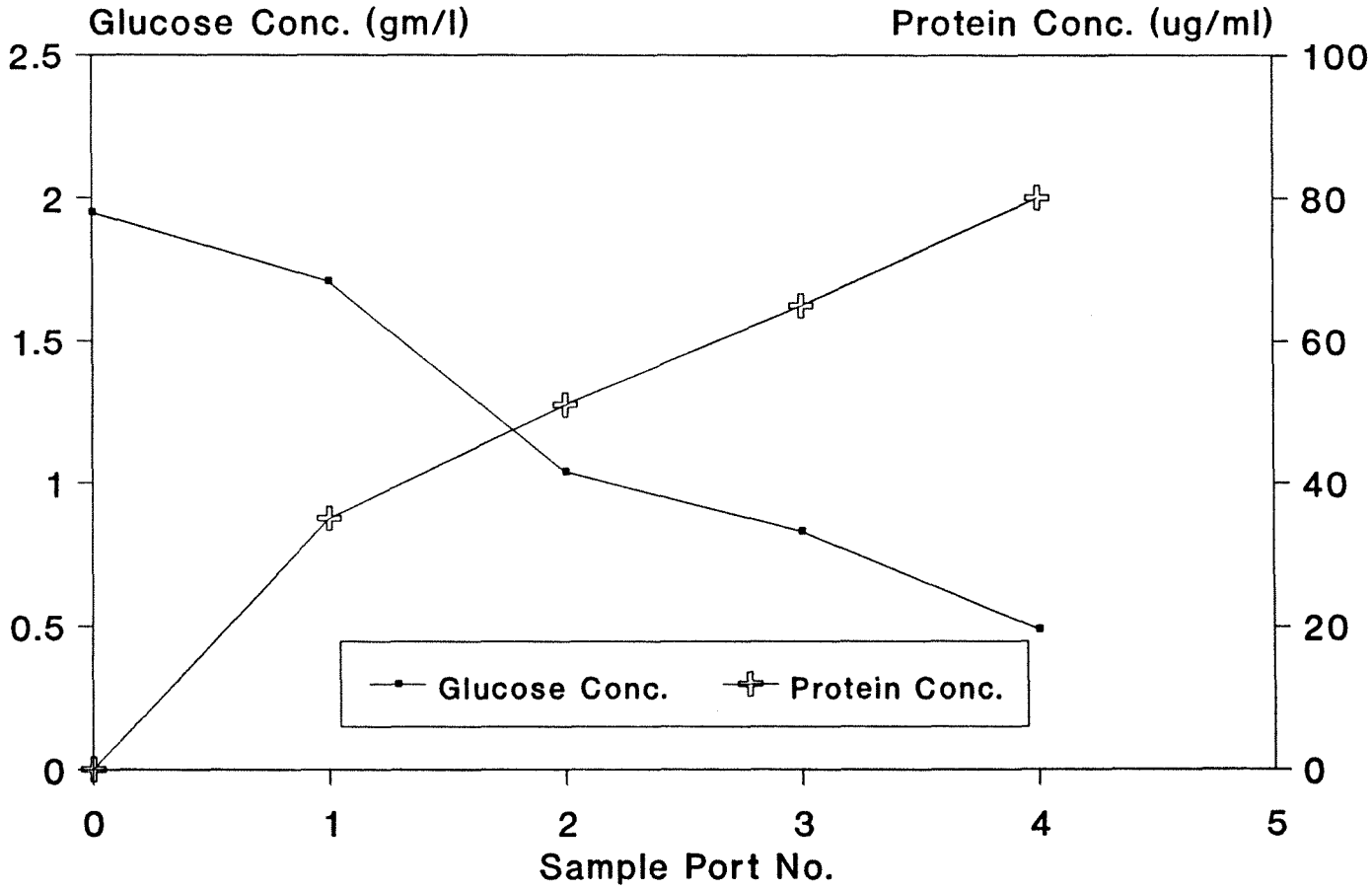

Figure 5

Change in pH in Packed Bed Reactor

Flow Rate = 2 ml/min

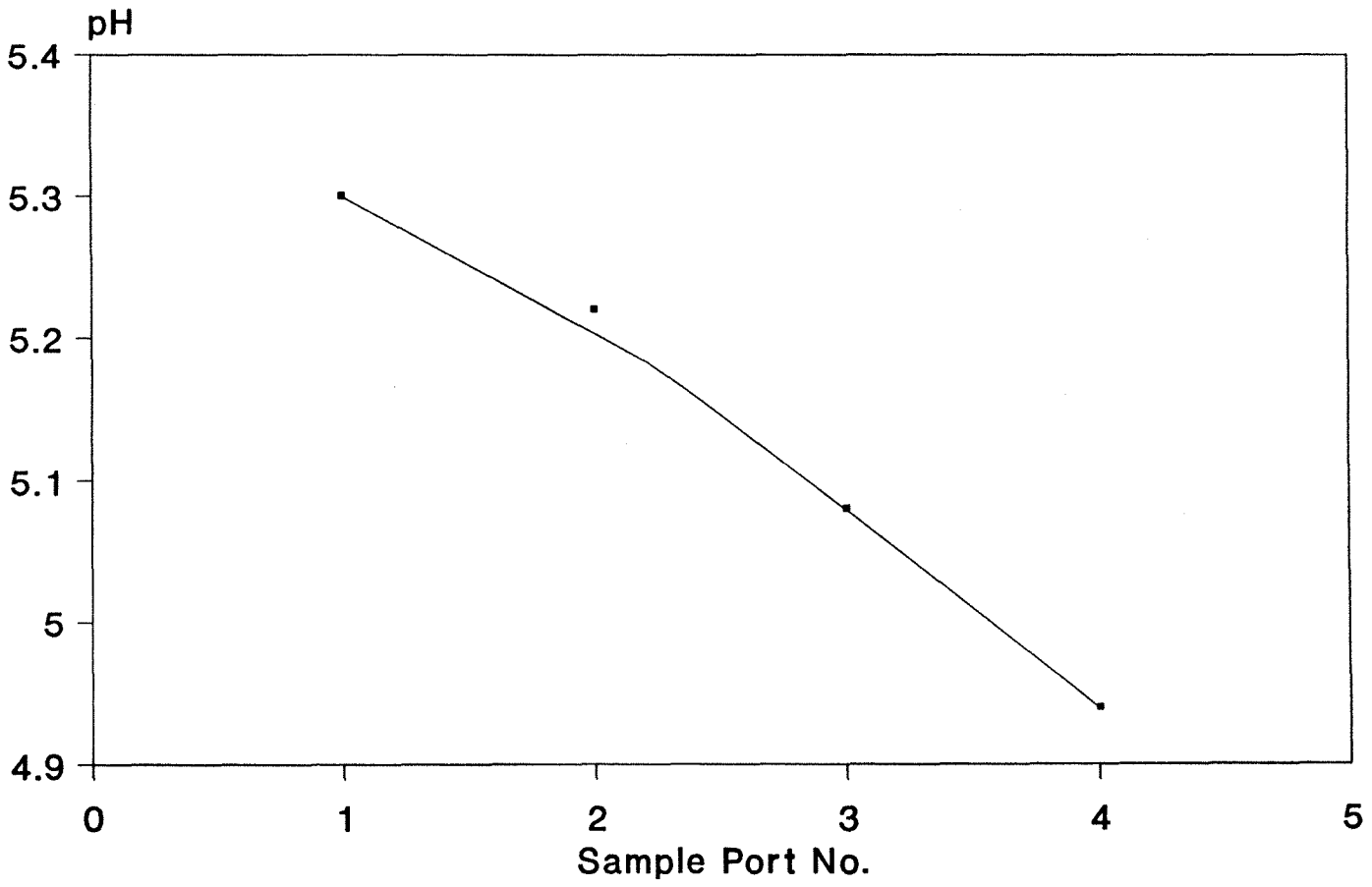

Figure 6

Biodegradation of Lindane in Sand
Direct Inoculum
Initial Glucose : 10 gm/kg of sand

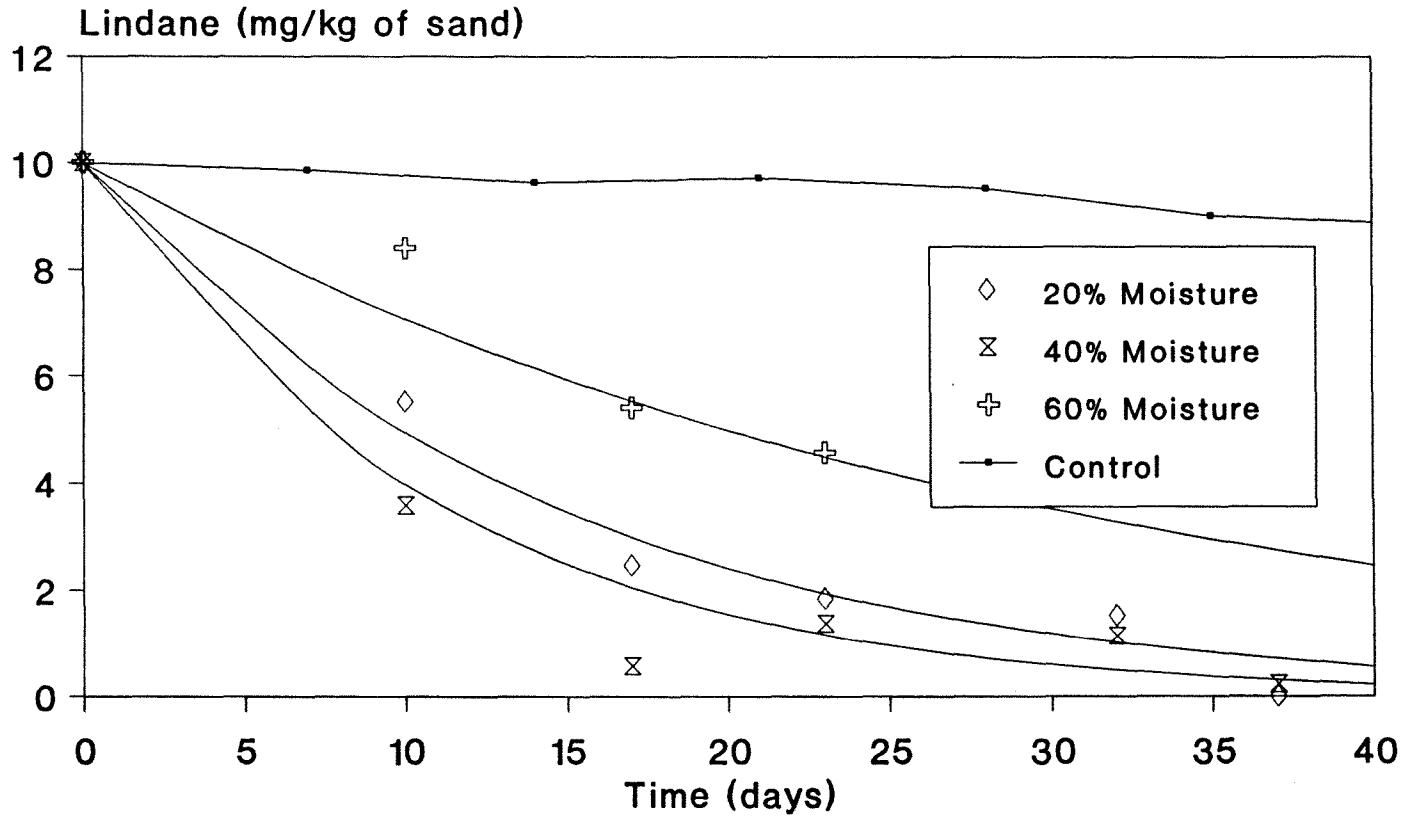

Figure 7

Biodegradation of Lindane in Sand
Direct Inoculum
Initial Glucose : 20 gm/kg of sand

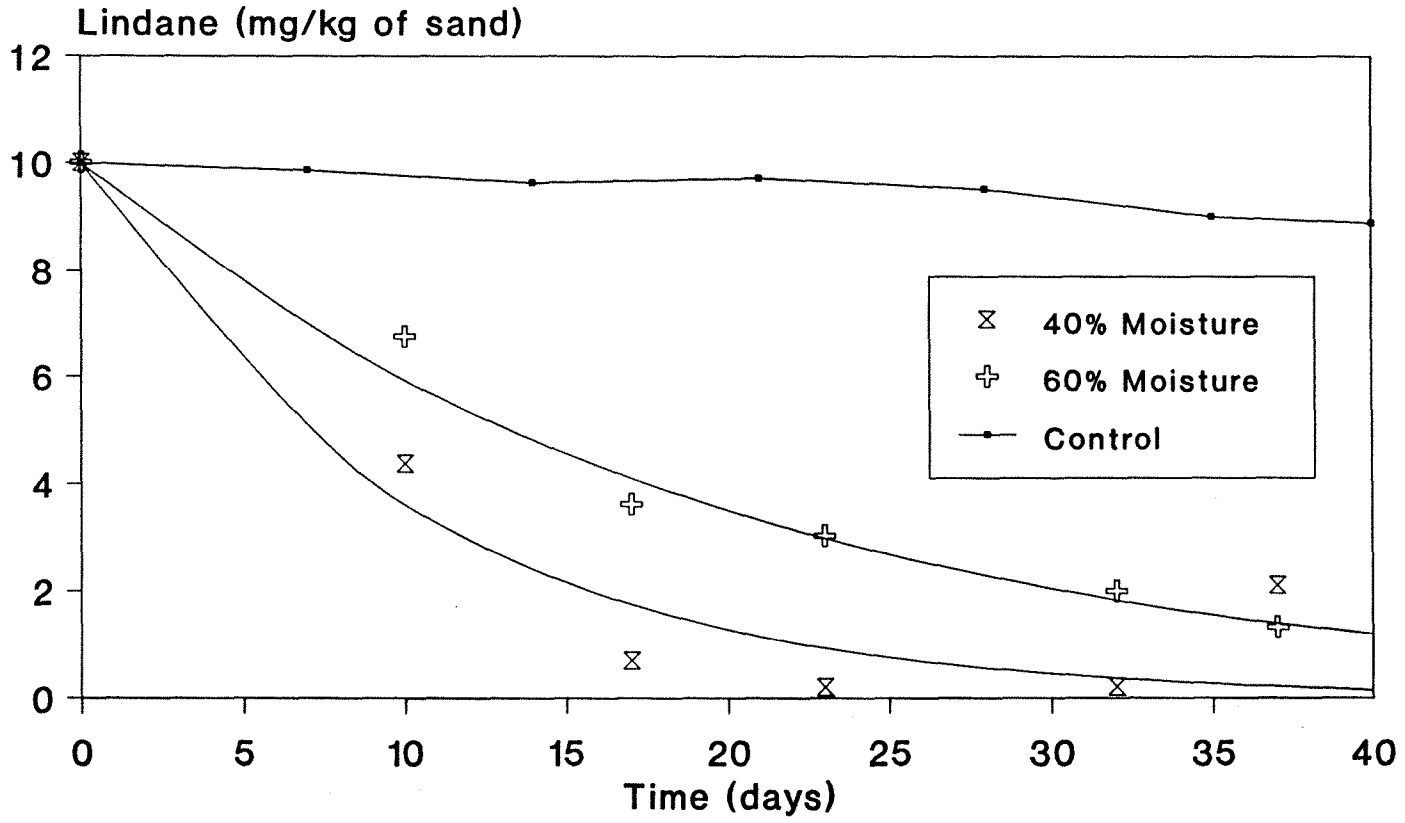

Figure 8

Biodegradation of Lindane in Sand
Inoculum- Fungus grown on wood chips
No Carbon Source

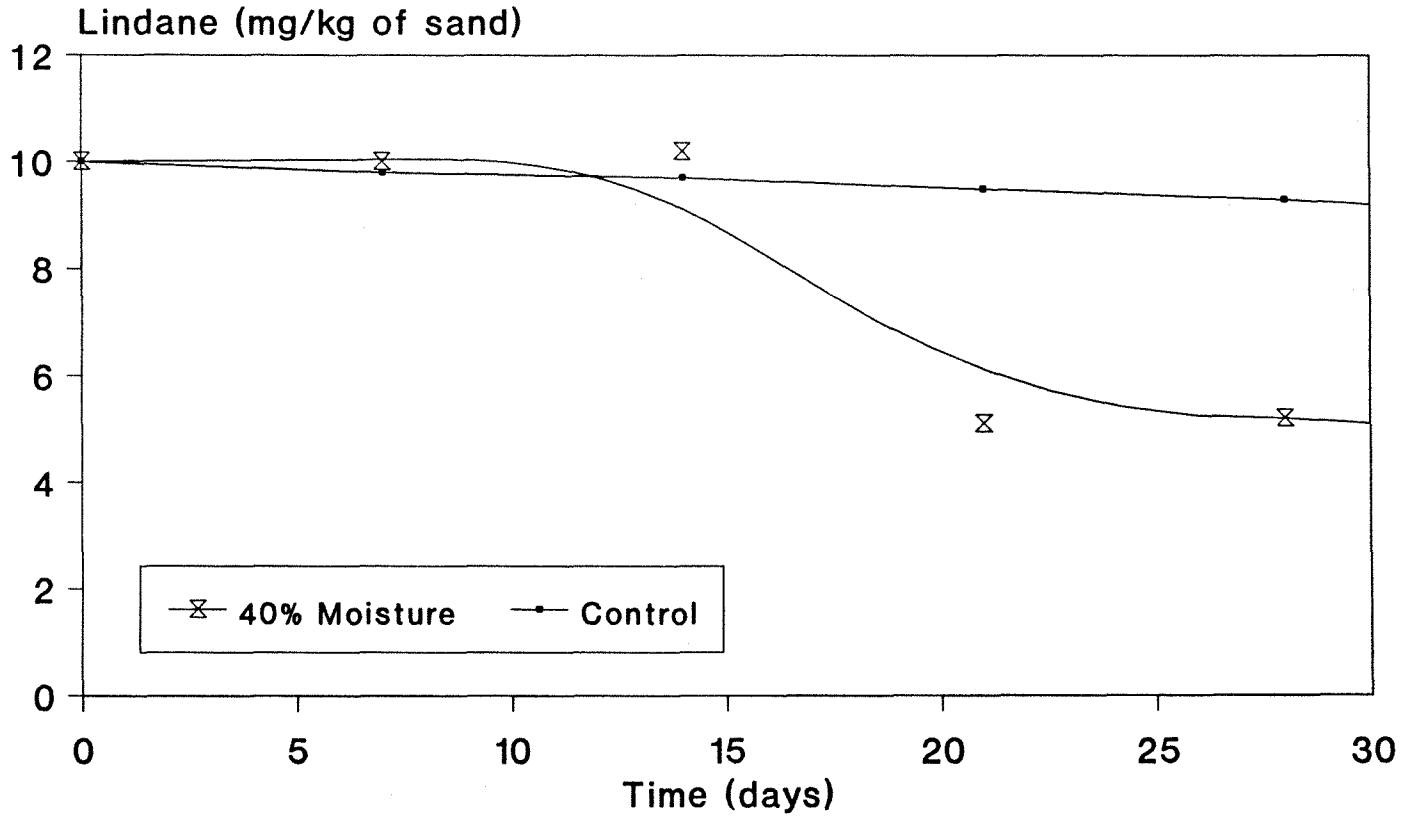

Figure 9

Biodegradation of Lindane in Sand
Inoculum- Fungus grown on wood chips
Initial Glucose : 10 gm/kg of sand

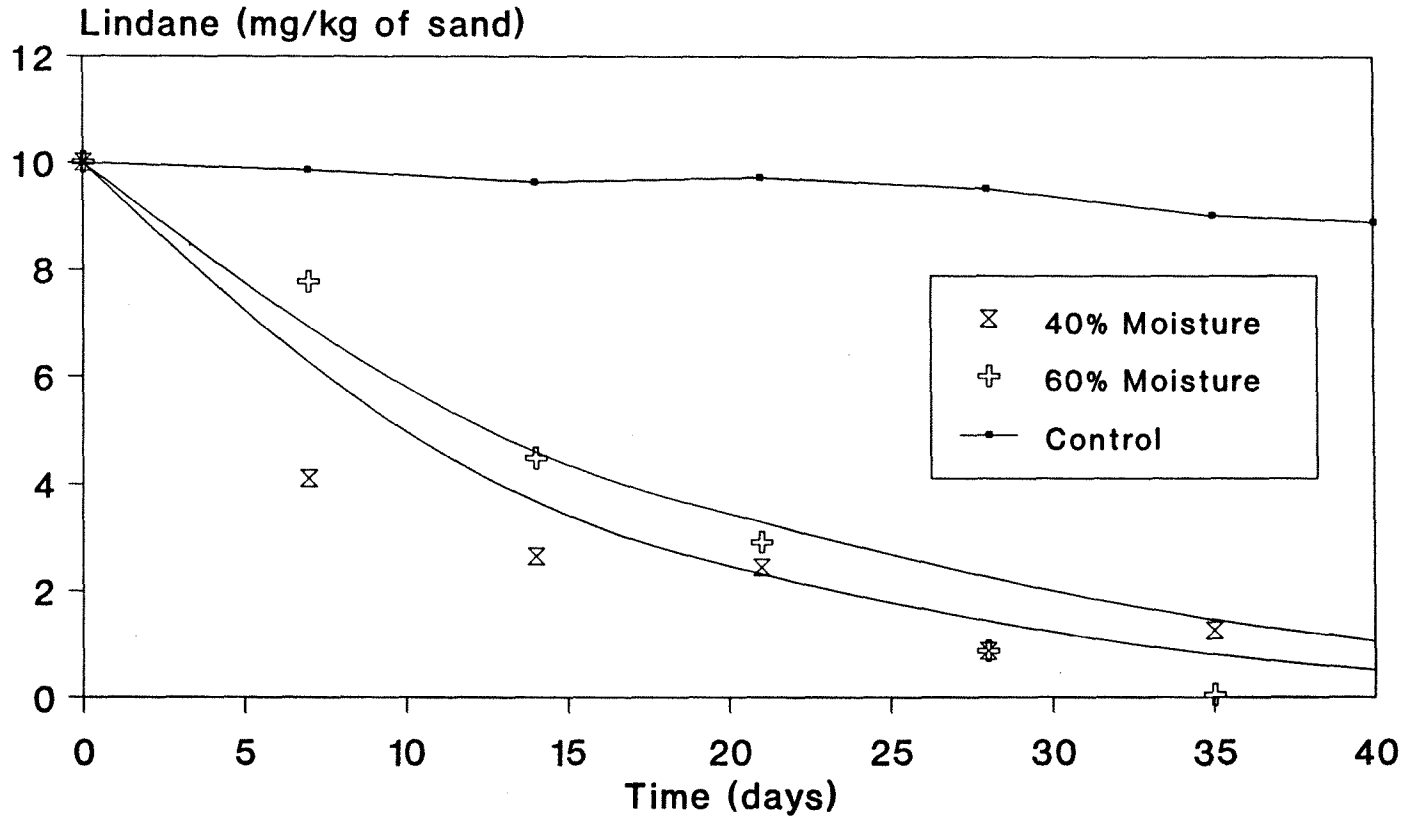

Figure 10

Appendix 1

Use of Residence Time distribution (RTD) for the Determination of Type of Flow Through Reactor

The elements of fluid taking different routes through the reactor may require different lengths of time to pass through the reactor. The distribution of these times for the stream of fluid leaving the reactor is called the exit age distribution *E*, or the residence time distribution RTD of fluid. With no tracer initially present anywhere, if a step input of tracer of concentration C_0 on the fluid stream entering the reactor is imposed, then a time record of tracer in the exit stream from the reactor, measured as C/C_0 is gives the F curve. (Levenspiel, Page 259)

Mathematically,

$$E = dF/dt$$

Thus, from the observation of the residence time distribution experiment we can generate an F-curve by dividing the actual absorbance value with the absorbance of solution at the inlet of the reactor. *E*-curve can be generated from F-curve by taking derivatives at different points. With the help of *E*-curve one can perform following statistical calculations in order to find the extent of axial dispersion in the reactor.

Average residence time can be defined as,

$$\bar{t} = \int tE\,dt$$

or,

$$\bar{t} = \sum t_i E_i \delta t$$

$$= 4.88 \text{ hrs.}$$

Similarly,

$$\bar{t^2} = \sum t_i^2 E_i \delta t$$

$$= 24.418 \text{ hrs.}$$

The spread of the distribution can be defined as,

$$\sigma^2 = \int (t - \bar{t})^2 E dt$$

or, .

$$\sigma^2 = t^2 - (\bar{t})^2$$
$$= 24.418 - (4.88)^2$$
$$= 0.6036 \text{ hr}^2$$

$$\sigma_\theta^2 = \frac{\sigma^2}{\bar{t}^2}$$
$$= 0.0253$$

$$\sigma_\theta^2 = 2(D/ul) - 2(D/ul)^2(1 - e^{-ul/D})$$

By trial and error,

$$D/ul = 0.012 = 0.01$$

Which suggests that the flow through the packed bed reactor used in this study behaves like an ideal plug flow reactor with negligible dispersion.

Table 1A
RTD in Packed Bed Reactor
Flow Rate 2 ml/min
Sample Port No. 2

Time hrs.	Absorbance	Concentration g/l
0.0	0.0	0.0
1.0	0.0	0.0
2.0	0.0	0.0
3.0	0.0	0.0
3.5	0.0	0.0
4.0	0.0	0.0
4.25	0.02	0.0013
4.5	0.07	0.0048
4.75	0.25	0.017
5.0	0.84	0.058
5.25	1.1	0.076
5.5	1.35	0.093
5.75	1.40	0.096
6.0	1.40	0.096
6.5	1.42	0.098
7.5	1.40	0.096

RTD in Packed Bed Reactor

Flow Rate = 2 ml/min

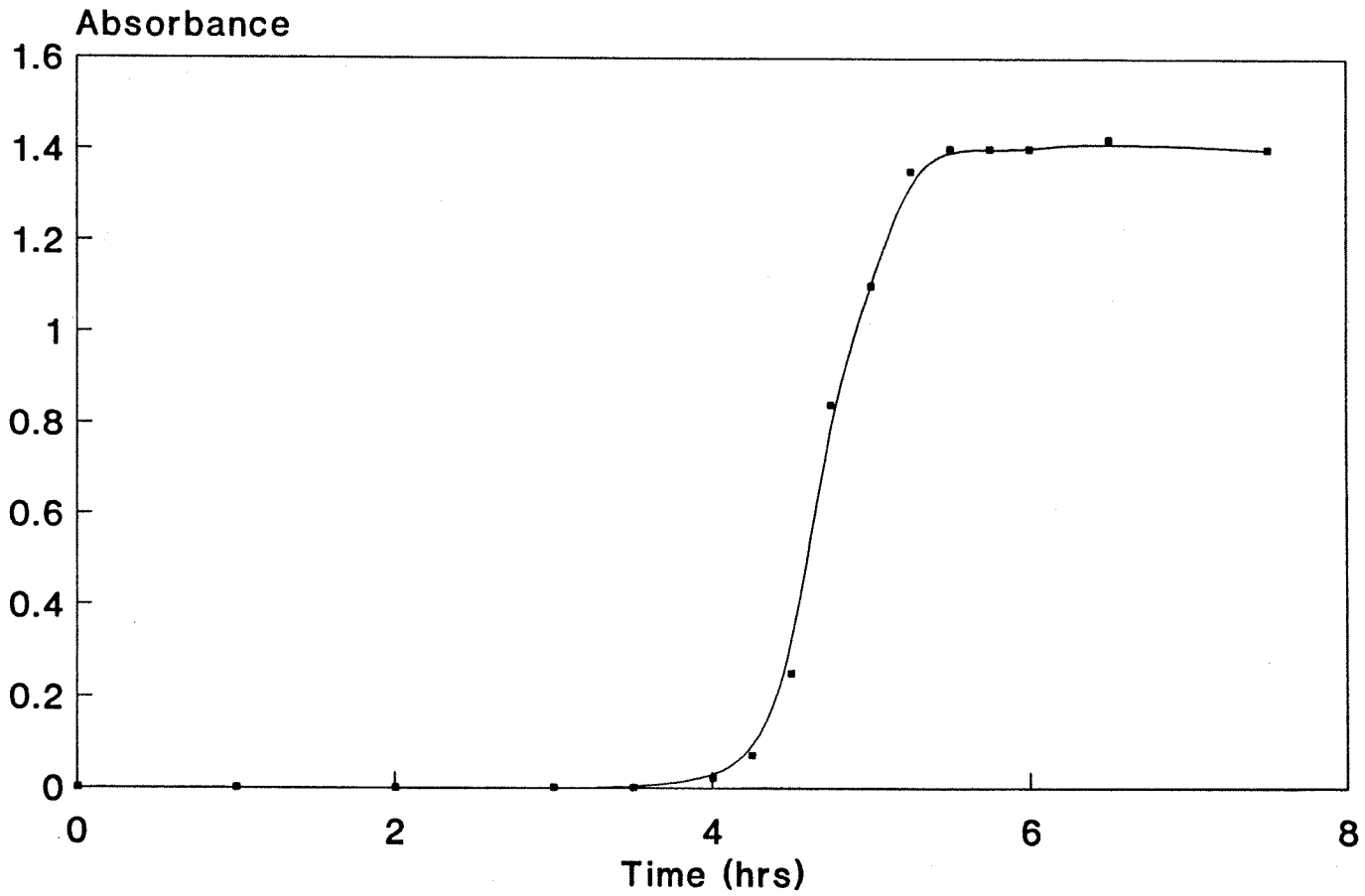

Figure 1A

RTD in Packed Bed Reactor

F-Curve

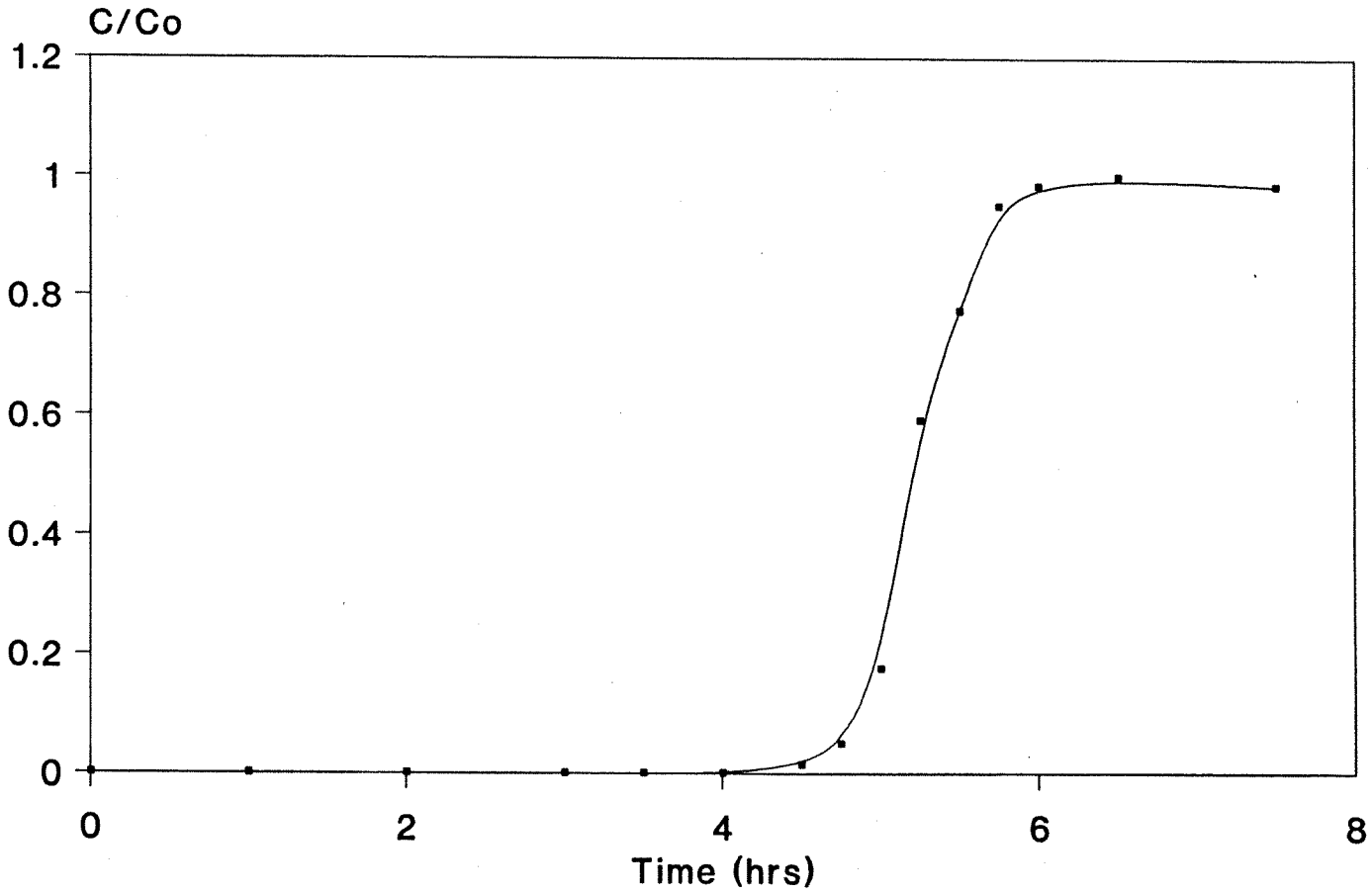

Figure 2A

RTD in Packed Bed Reactor

E-Curve

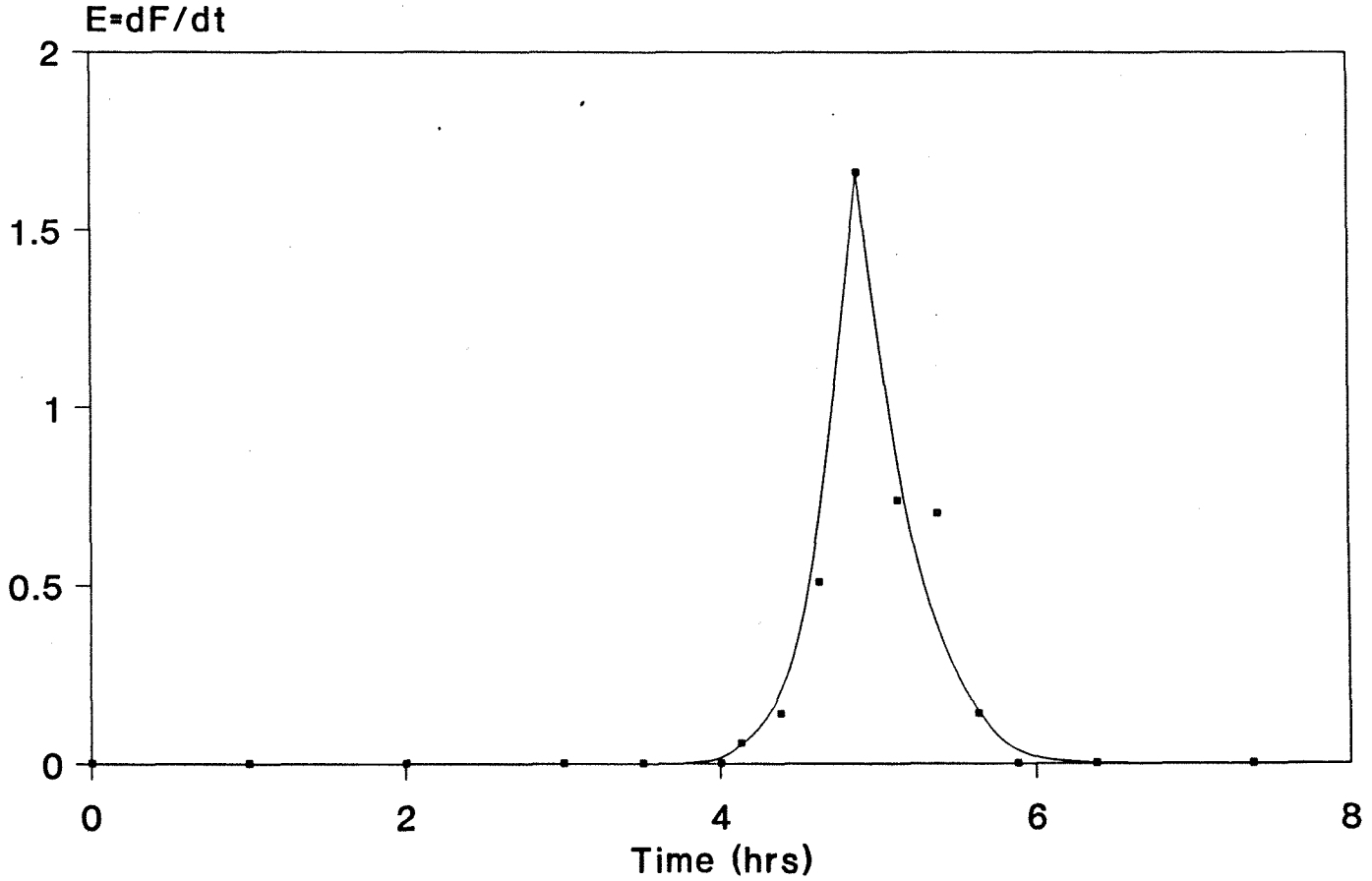

Figure 3A