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# Recalcitrance of polychlorinated biphenyls to biodegradation by phanerochaete chrysosporium in soil

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# RECALCITRANCE OF POLYCHLORINATED BIPHENYLS TO BIODEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM IN SOIL

by

**Thierry B. Poncet**

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 Master of Science in Environmental Science
1992

## Approval Sheet

Title of Thesis

Recalcitrance of Polychlorinated Biphenyls to Biodegradation by *Phanerochaete chrysosporium* in Soil

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**Abstract**

Title of Thesis Recalcitrance of Polychlorinated Biphenyls to Biodegradation by *Phanerochaete chrysosporium* in Soil

By Thierry B. Poncet

Master of Science in Environmental Sciences (1992)

Thesis directed by Dr. Piero M. Armenante

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---

The white rot fungus *Phanerochaete chrysosporium* has been shown in the past to effectively degrade a number of chlorinated aromatic compounds. Previous investigators (Eaton, D. C., "Mineralization of Polychlorinated Biphenyls by *Phanerochaete chrysosporium*, a Lignolytic Fungus", *Enz. Microb. Technol.*, **7**, 194-196 (1985); Bumpus, J. A., Tien, M., Wright, D., and Aust, S. D., "Oxidation of Persistent Environmental Pollutants by White Rot Fungus", *Science*, **228**, 1434-1436 (1985)) have also claimed that this fungus is capable of mineralizing polychlorinated biphenyls (PCBs) in submerged cultures under stationary conditions. Therefore, the objective of this work was to study the biodegradation activity of the fungus in soils contaminated with PCBs. The results obtained so far indicate however that no significant degradation of PCBs in contaminated soil or sand occurs. Several types of experiments were conducted in which different parameters were varied: type of PCBs, amount and type of inoculum, type of solid support matrix, and concentration of some key nutrients. In most of the experiments, the amount of PCB recovered from the soil after the exposure to the fungus was lower than the initial amount. However, the relative concentrations of the PCBs congeners before and after the fungal treatment was the same in both cases. Since biodegradation typically results in the attack on some preferential congeners of the PCB mixture rather than a uniform, across-the-board mineralization, the results obtained so far appear to indicate that the fungus does not biodegrade PCBs. Furthermore, experiments in which the fungus was exposed to soil contaminated with 2,4,6-trichlorophenol (TCP), used as control experiments, showed that, in sand, this compound was degraded, whereas under the same conditions, PCBs would not undergo any degradation at all. In similar experiments conducted in soil, no degradation of PCBs was detected, but it is difficult to determine if degradation of TCP had occurred.

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# Chapter I INTRODUCTION

There are at least 10,000 potential Superfund sites throughout the United States. Contamination of these sites is the result of prior uncontrolled handling and disposal of hazardous substances, or other unregulated waste management practices. The United States government is very concerned with the remediation of these sites, and one of the main goals of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) is to clean up these sites (Stoll, 1990). When evaluating a site, the presence of populations or potentially contaminated water are very much taken into account to decide which remedial actions could be taken. The toxicity and the stability of the compounds present in the site are also important factors in this evaluation.

PCBs are a class of Hazardous Compounds frequently found in Superfund Sites. The U.S. Environmental Protection Agency (USEPA) (1985) estimates that 2,500 sites in the United States are highly contaminated with PCBs. Between 1929 and 1977, it is estimated that about 540,000 metric tons of PCBs were produced in the US and an estimated 350,000 metric tons are still in use. For many years, prior to the introduction of more environmental safe regulations, the state of the art in

the elimination of PCBs has been landfilling. Therefore, it is not surprizing that many Superfund sites in the US are still contaminated with PCBs.

Biological treatment is a potential treatment alternative for some of the Superfund sites. The white rot fungus *Phanerochaete chrysosporium* is one of the microorganisms which could be used to biodegrade some recalcitrant chemicals. This fungus belongs to a family of wood-rotting fungi that is found all over the Northern Hemisphere. This fungus decomposes wood by breaking down lignin, a complex O-methylated polymer of phenolic hydroxylgroups that is otherwise very resistant to decay. The extracellular lignin-degrading enzymes serve to break down the lignin structure into smaller molecules that can be assimilated by the fungus. The intracellular enzyme components complete the conversion of the lignin fragments into carbon dioxide (Glaser et al., 1989).

Since lignin is an irregular macromolecule, the enzyme systems that degrade lignin must be non-specific. *P. chrysosporium* produces at least 10 extracellular hemoproteins and roughly half have ligninase activity (Glaser et al., 1989). Some of the more common substructures of lignin resemble the chemical structure of many persistent organic compounds contaminating the environment. These structural similarity gave sufficient reasons to pursue the application of the white rot fungus, *Phanerochaete chrysosporium* to accomplish the biodegradation of hazardous waste constituents.

It has already been shown that under laboratory conditions, *P. chrysosporium* can degrade a number of compounds in aqueous solutions such as DDT, polychlorinated dibenzo(p)dioxin and chlorophenols (Bumpus et al., 1985; Faison et

al., 1985; Lewandowski et al., 1990, Tien et al., 1984). Researchers are looking for other hazardous chemicals that could be degraded by *P. chysosporium*.

However, there is little information about the ability of microorganisms to carry any kind of biodegradation activity on PCBs, and also very little information about the ability of this fungus to be active in soil. Therefore, the purpose of this research work is to determine *P. chrysosporium*'s ability to degrade PCBs in soil. In addition, the biological degradation activity of the fungus has also been tested against 2,4,6-Trichlorophenol (TCP) in soil. These experiments not only served to determine the biodegradability of TCP in soil by the fungus but were also used as control experiments for the PCB experiments, in order to show the comparative ability of the fungus to degrade the two classes of compounds (PCBs and TCP) under the exact same conditions.

# Chapter 2 LITERATURE REVIEW

## 2.1. POLYCHLORINATED BIPHENYLS

### 2.1.1. Introduction

PCBs are synthetic chemicals that were first synthesized in 1881. Their industrial production began in 1929. Since then, PCBs have been widely used in many industrial applications. Because of their remarkable electrical insulating capacities and their non flammable nature, they soon gained widespread use as coolants and lubricants in transformers and electrical equipments where these properties were essential. PCBs replaced combustible insulating fluids. They were also used in the manufacture of a wide variety of common products, such as plastics, adhesives, paints, varnishes, carbonless copying paper, newsprints, fluorescent light ballasts and caulking compounds (Whelan, 1981).

### 2.1.2. PCB Chemical Structure

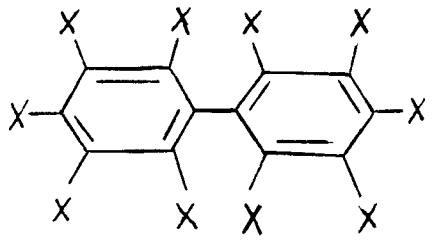
PCBs are chemical compounds whose molecule consists of two aromatic rings and two or more chloride atoms (Figure 1). They form a family of two hundred and nine congeners (Bedard, 1989). Commercially, they are found in mixtures of these congeners. Depending on the nature and quantity of these congeners, they were sold under different names such as (Wagner, 1985):

- Aroclor
- Clophen
- Fenclor
- Kanoclor
- Phenoclor
- Pyralene
- Santotherm

Polychlorinated biphenyls and polychlorinated triphenyls were primarily commercialized under the name "Aroclor". To distinguish between the different types of Aroclor, a four digit number code was established.

The first two digits indicate if the mixture is made of biphenyls or triphenyls. If these first two digits are 12, the Aroclor is a mixture of biphenyls. If these first two digits are 54, then this Aroclor is a mixture of triphenyls. If the first two digits are 25 or 44, then the Aroclor is a mixture of biphenyls and triphenyls (Wagner, 1985).

Figure 1 - General structure of PCBs



Where X is wether a chlorine or an hydrogen

The last two digits indicate the percentage in weight of chlorine in the solution. For example, Aroclor 1254 is a mixture of biphenyls that contains 54 % in weight of chlorine while Aroclor 1260 is a mixture of biphenyls containing 60 % of chlorine.

### 2.1.3. Physical Properties of PCBs

Table 1 gives the main physical properties of Aroclor 1254 and Aroclor 1260. PCBs are very lipophilic. Their boiling points are high and their vapor pressures are low. Because of their liposolubility, and high stability, these molecules have the tendency to accumulate in the environment and in living organisms.

### 2.1.4. PCB Toxicity

The first evidence of the toxicity of chlorinated compounds was observed in the 1930's as workers exposed to different type of chemicals, including PCBs, developed chloracne (severe skin rash). The carcinogenic effects of PCBs on animals (rats, mice) have been established. However, the same effects have never been proved on human beings (Whelan, 1985). PCBs are classified $B_2$ by the EPA (1989), which indicates that there is sufficient evidences of carcinogenicity on animals but inadequate or no evidence on human.

As it was shown in animal experiments, PCBs are lethal at higher dosage. The Material Safety Data Sheet gives the Lethal Dose 50 (LD50) for rats as:

- 1295 mg/kg for Aroclor 1254
- 1315 mg/kg for Aroclor 1260.

**Table 1 Physical Properties of Aroclor 1254 and 1260 (Whelan, 1985)**

| | Aroclor 1254 | Aroclor 1260 |
|---|---|---|
| Melting Point | 10°C | - |
| Boiling Point | 365-390°C | 385-420°C |
| Specific Gravity | 1.505 | 1.57 |
| Solubility in Water | Not Soluble | Not Soluble |
| Phase | Oily Fluid | Wax |
| Vapor Pressure at 38°C | 0 mm Hg | 0 mm Hg |

The routes of exposure are:

- Inhalation of fumes or vapors
- Ingestion of contaminated food or soil
- Skin contact

### 2.1.5. Causes of Contamination

The fabrication and use of PCBs were banned in the US in 1979 by the Federal "Toxic Substances Control Act" (TSCA) (Wentz 1989). Many European countries have banned PCBs also. However, the causes of contamination in the environment remain. The principal sources of contamination are the following:

- Leak from former transformers and capacitors
- Combustion of some plastics
- Leak from PCB storage area or industrial waste

### 2.1.6. PCB Regulation

The regulation of PCBs is an anomaly in that, nowhere else in the environmental law is a substance banned or phased out by name (Marshall, 1990). This unusual step phased out some uses of PCBs in 1977 and most productions and uses in 1979.

By October 1977, no one was allowed to manufacture, process, distribute, or use any PCBs except in a "totally enclosed manner" defined as any manner that will ensure no exposure to human beings or the environment." Utilities and other owners of PCB-filled electric transformers and capacitors were permitted to maintain their equipments for its working life, provided it did not leak or require

major servicing. Any transformer that was not totally enclosed had to be banned (Marshall, 1990).

### 2.1.7. PCB Incineration

PCBs can be incinerated. However, they are very resistant to incineration. During combustion, they break down into CO, $CO_2$, HCl, phenols, and other toxic compounds, depending on the temperature. If the temperature is around 600-650$^{o}$C, and in the presence of an excess of oxygen, PCBs will produce polychlorodibenzofurane (PCDF) and polychlorodibenzodioxins (PCDD). Because of the highly toxicity of these compounds, PCB incineration is regulated under the Toxic Substances Control Act (TSCA). TSCA Incineration Standards are described below:

- The PCBs introduced in the incinerator must be maintained for 2 second at 1200$^{o}$C and 3% excess oxygen in the stack gas. An alternative is to maintain the PCBs for 1.5 second at 1600$^{o}$C and 2% excess oxygen in the stack gas. The Destruction and Removal Efficiency (DRE) has to be more than 99.9999 percent.

- Monitoring of the stack emission products must be conducted when an incinerator is first used for the disposal of PCBs. The following exhaust emissions must be monitored :

- Oxygen
- Carbon Monoxide
- Oxides of Nitrogen
- Hydrogen Chloride
- Total Chlorinated Organic Content

- PCBs

- Total Particulate Matter.

### 2.1.8. Biological Treatment

Bedard et al. (1989) have reported that polychlorinated biphenyls are undergoing biodegradation in nature. The analysis of PCBs extracted from Hudson River sediments have shown that their GC fingerprints were markedly different from the commercial mixture originally discharged. It was found that there was a relative peak height variation between the 2 chromatograms (the sample from the Hudson river and the commercial mixture used as a control). Initially, no reasons for the variation could be given. Later, the GC patterns of PCB samples obtained from the environment were compared with those of commercial PCBs after exposure to an anaerobic culture of known bacteria in the laboratory. The same patterns were observed for the 2 chromatograms. This implied that some of the compounds present in the PCB mixture were biodegraded by these organisms. The degradation occurred under laboratory conditions and in the environment (Bedard, 1989).

Examples of chromatograms showing PCB biodegradation are presented in Figure 2a and 2b. Figure 2a shows 3 chromatograms. Chromatogram A is from a control experiment in which no biomass was added. Chromatogram B represents the same PCBs after addition of one unit dose of organisms. One can see that the height of some of the peaks decreased drastically, whereas other peaks kept the size they had in the control chromatogram. Chromatogram C shows the PCB after the addition of 6 consecutive unit doses of organisms. The decrease in the peak heights is clearly more pronounced.

**Figure 2a - Example of PCB degradation**
**A - Control (No degradation)**
**B - PCBs after exposure to 1 dose of organisms**
**C - PCBs after exposure to 6 consecutive doses of organisms**

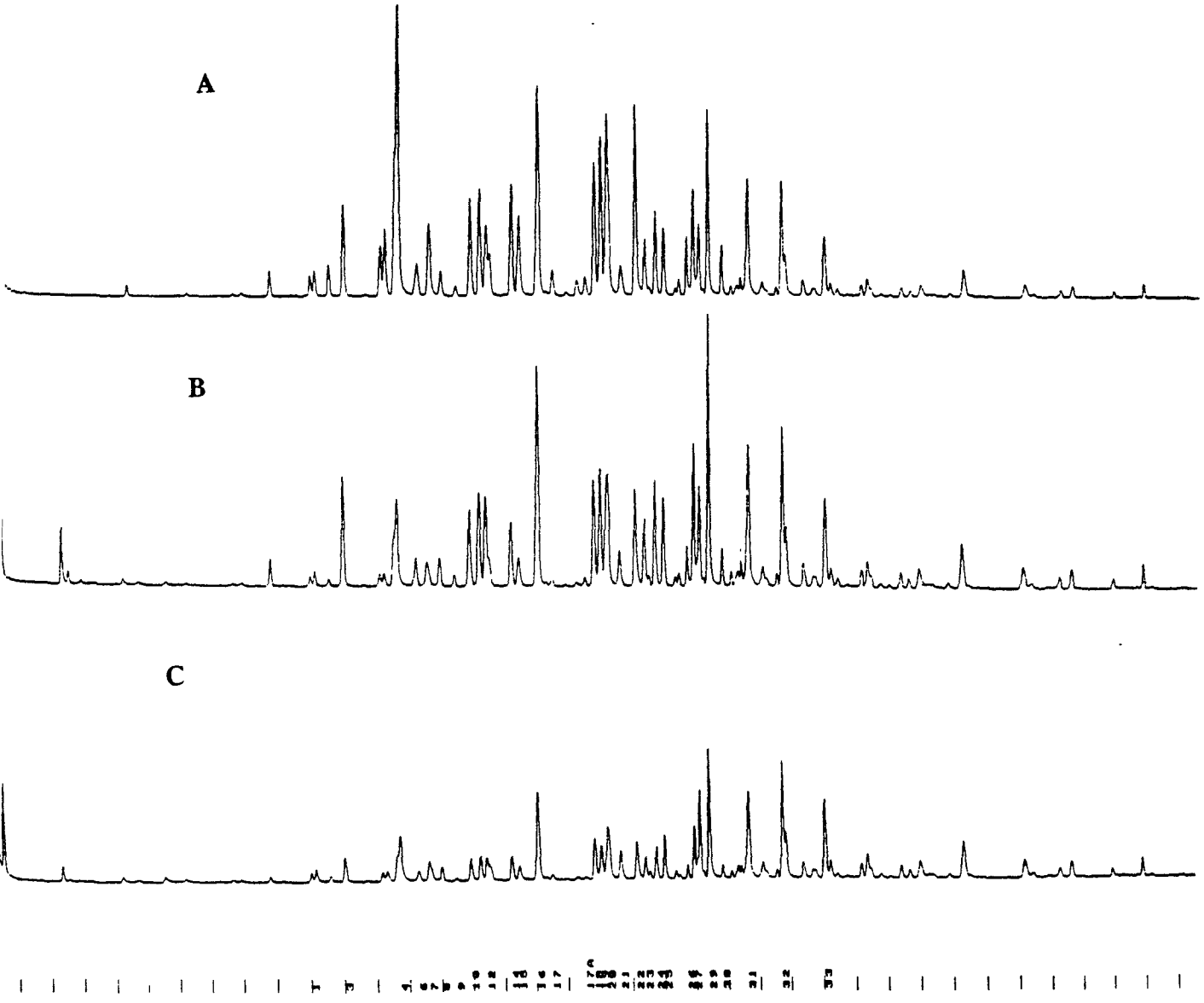


**Figure 2b - Example of PCB degradation**
**A - Control (No degradation)**
**B - PCBs after exposure to an given organism**

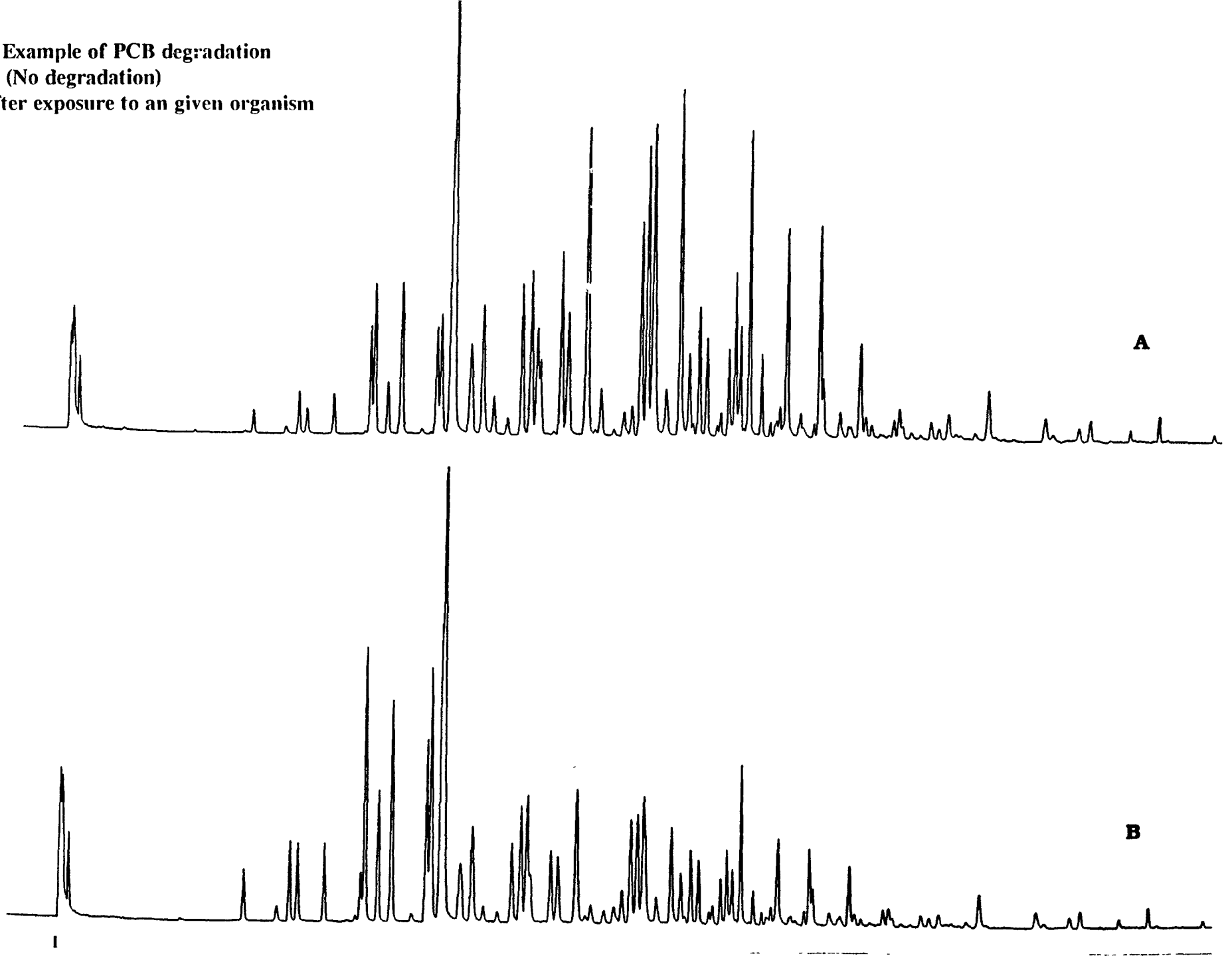


Figure 2b includes the chromatogram (A) for a control experiment and the chromatogram (B) of the same PCB mixture exposed to an organism (different from that used for Figure 2a). The relative peak heights in the chromatograms are not the same. This means that in Chromatogram B some peak decreased in height whereas others maintained the same size they had in the control. Again, this is evidence of biodegradation.

## 2.2. PHANEROCHAETE CHRYSOSPORIUM

*P. chrysosporium* is a common wood rotting fungus that utilizes wood cellulose as its primary carbon source. In order to access cellulose, it must degrade lignin. Since lignin is a naturally occurring highly complex polymer very resistant to attack by most organisms, the fungus secretes a hydrogen peroxidase-dependent, extracellular, lignin-degrading enzyme system capable of breaking down the lignin structure. The ability of the fungus to degrade the complex structure of lignin suggested that recalcitrant organohalides having a very stable benzene nucleus may also be degraded by this fungus (Glaser et al., 1989).

Several papers have described the ability of this organism to mineralize a wide spectrum of chlorocarbons, such as DDT, Polychlorinated dibenzo(p)dioxins, chlorinated alkanes and chlorophenols (Bumpus et al., 1985; Faison et al., 1985; Lewandowski et al., 1988; Tien et al., 1984). As a result, this fungus holds a great deal of interest as a biological tool in hazardous waste management.

Many studies have been carried out to investigate the feasibility of using *Phanerochaete chrysosporium* for the treatment of selected xenobiotics in liquid culture (Bumpus et al., 1985; Eaton, 1985). However, the fungus' ability to degrade xenobiotics in soil has received much less attention. Ajaykumarh (1989) found that *P. chrysosporium* has the ability to degrade lindane in sand.

### 2.2.1. Degradation of Waste Water Contaminants by P. chrysosporium

Arjmand et al. (1985) had obtained 35% to 59 % mineralization of 4-chloroaniline and 3,4-dichloroaniline, respectively. Bumpus et al. (1985) have 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 $CO_2$. Pellinen et al. (1988) studied the dechlorination of high-molecular-weight chlorolignin and obtained almost 50 % in the total organic chlorine content of chlorolignin during one day of treatment. Mileski et al. (1988) showed that the time of onset, time course and decline in the rate of pentachlorophenol mineralization was similar to those observed for lignin degradation. Sanglar et al. (1986) showed that the fungus oxidized benzopyrene to $CO_2$. Pak (1988) found that *P. chrysosporium* can degrade 2-chlorophenol. Zitzelsberger et al. (1987) reported that *P. chrysosporium* was able to degrade veratrylglycerol-2,4-dichlorophenyl ether, a lignin bound xenobiotic residue.

Leatham et al. (1983) studied POL 88, a mutant strain of *P. chrysosporium*, and found that it can degrade different aromatic compounds. Thirty-six compounds were tested. Sixteen of them degraded at least 20 % in three days. This fungus is a very versatile and a non-specific degrader of aromatic molecules.

## 2.2.2. Parameters Affecting the Biodegradation of Organic Contaminants in Waste Water

The parameters affecting the biodegradation of xenobiotic compounds by the fungus are not very well known. However, the degradation of lignin has been studied extensively, and it is believed that degradation of chlorinated aromatics follows a similar path (Haq, 1989). Various parameters for lignin degradation have been studied and are listed below.

### 2.2.2.1. Effect of Nitrogen

The nitrogen concentration in the medium is a critical factor for the lignolytic activity (Bumpus et al., 1990, 1985; Eaton 1985; Faison et al., 1985, 1986; Reid et al., 1983)

Reid et al. (1983) studied the effect of nitrogen sources and supplements on degradation of aspen wood lignin. The addition of nitrogen strongly inhibits the degradation of lignin. However, 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 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 24 mM of nitrogen than in one containing 2.4 mM of nitrogen.

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.

#### 2.2.2.2. Effect of Oxygen

Since lignin degradation has been shown to be an oxidative process, the concentration of oxygen is a crucial parameter. Kirk et al. (1986) maintained cultures under a one-atmosphere gas phase that contained 5%, 21%, and 100% oxygen. The growth during the first three days of incubating was similar to all three $O_2$ concentration. Lignin decomposition began after the initial growth period under 21 % and 100 % $O_2$ but not under 5 % $O_2$.

#### 2.2.2.3. Effect of pH

Kirk et al. (1986) found that the optimum pH for fungal growth was 5.5. For the lignin degradation, the optimal pH was 4.5 and a reduction in activity takes place for pH lower than 3.5 and above 5.5.

#### 2.2.2.4. Effect of Carbon Source

Kirk et al. (1986) demonstrated that any of several carbohydrates can serve as growth substrats 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 xenobiotics mineralization were achieved for at least 90 days when complex carbohydrates (i.e., cellulose and starch) were used as carbon source. Reid et al. (1983) showed that mineralization stopped in those cultures whose carbon as energy source had been depleted.

#### 2.2.2.5. Effect of Agitation

The effect of agitation has also been studied (Faison et al., 1985; Jager et al., 1985; Leisola et al., 1985; Reid 1983). Most of the studies have shown that agitation reduces 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.

#### 2.2.2.6. Effect of Temperature

Glaser et al. (1988) have shown that the white rot fungus can grow in a wide range of temperatures. The growth rate increases as the temperature increases from 10 to 39$^{o}$C. According to Kirk et al. (1978), the optimal growth temperature is 39$^{o}$C.

### 2.2.3. Biodegradation of Organic Contaminants in Soil by P. chrysosporium

The general success of the liquid phase degradation has encouraged the use of the fungus in soil. However, very few papers are available on the activity of the fungus in soil.

Lamar et al. (1989) have tried to degrade pentachlorophenol (PCP) in soil inoculated with *Phanerochaete chrysosporium*. They measured the mineralization and volatilization of PCP, as well as its transformation products, over a two month period. They observed a decrease of 98% in the extractable concentration in inoculated soils compared to the uninoculated soil.

Bumpus et al. (1990) studied degradation of DDT and selected PAHs in soil. They obtained respectively 11% and 7.7% degradation.

## 2.2.4. Parameters Affecting Degradation of Organic Contaminants in Soil by P. chrysosporium

### 2.2.4.1. Effects of Carbon Sources

Since *Phanerochaete chrysosporium* cannot 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.

Lamar et al. (1990) in there study of PCP degradation in soil obtained the highest rate of degradation in masham soil. They suggested that the differences in degradation rate might be the consequences of the quantity of nutrients present in these soils.

### 2.2.4.2. Effect of pH

Bumpus et al. (1990) carried out several experiments with $^{14}$C-DDT. They suggested that the optimum rate of degradation is between 3.5 and 6.0.

### 2.2.4.3. Effect of Temperature

Glaser (1989) found that the growth of the fungus in soil was unaffected between 25$^{o}$C and 35$^{o}$C, but significantly decreased above 39$^{o}$C.

## 2.3. Activity of P. chrysosporium against PCBs

The fungus has already been reported to degrade PCBs exposed to the fungus (Bumpus et al., 1985; Eaton et al., 1985). The paper by Eaton (1985) is particularly relevant since this author claimed that radiolabeled carbon dioxide was release by radiolabeled PCB. A closer examination of this work has shown that the amount of carbon dioxide released was only 10% of the stoichiometric amount of PCBs initially added. However, it is quite possible that part of this carbon dioxide could have been produced from the oxidation of more readily degradable impurities present in the PCB. This is typically the case for most radiolabeled materials. No assay on the purity of the PCBs were reported by the author. In addition, the chromatograms reported by Eaton are rather crude, do not show the multitude of peaks typically associated with Aroclors, and do not show any preferential decrease in the peaks of some of the congeners, as one would expect if a biological, or even a catalytic reaction would take place. Finally, the amount of PCBs added to the cultures in Eaton's work were always very small - typically between 0.44 ppb to 250 ppb.

Similarly, the paper by Bumpus et al (1985), indicates that only 1.1% of the initial amount of radiolabeled PCB was converted to radiolabeled $CO_2$. Once again, this could be easily attributed to the presence of easily oxidable impurities in the initial PCB. Furthermore, the concentration of PCB in the culture was also small, i.e. 36 ppb.

Identically extremely low concentrations but higher degradation rate of 11.5% were reported by Bumpus et al(1989).

The low degradation rates and the even lower concentration reported in these studies raise many doubt about the ability of *P. chrysosporium* to biodegrade PCB under laboratory condition.

# CHAPTER 3 MATERIALS AND METHODS

## 3.1.. MATERIALS

### 3.1.1. Soil

A soil sample provided by USEPA (Risk Reduction Engineering Laboratory, Release Control Branch, Edison, NJ) was used in most of the experiments conducted in this work. The soil was prepared by EPA by blending together the following components:

| Components | Weight % |
|---|---|
| Sand | 31 |
| Gravel N$^{O}$.9 | 6 |
| Silt | 28 |
| Topsoil | 20 |
| Clay | 15 |
| Montmorillonite(5%) | |
| Kaolinite(10%) | |
| | 100 |

The clean soil was analyzed by EPA and found to be free of anthropogenic organic contamination. The Total Organic Carbon (TOC) of the soil was found to be within the range 3-20 mg/kg. The cation exchange capacity of the soil averaged 133 meq/100g, and its natural pH was 8-8.5. The soil naturally contained small amounts (< 10 ppm) of arsenic, beryllium, cadmium, chromium, cobalt, copper, lead, nickel, and silver. Other elements found to be naturally present include aluminum (13,000 ppm), calcium (131,000 ppm), iron (10,300 ppm), potassium (1220 ppm), sodium (198 ppm), and zinc (33 ppm).

This EPA soil was sieved to get a more homogeneous soil and thus more homogeneous results. The sieve used was a U.S.A. Standard Testing Sieve No 25. The fraction below 710 um (Tyler equivalent 24 mesh) was used for the experiments.

### 3.1.2. Sand

The sand was purchased from a local retailer store. The sand was washed several times with water in order to remove organic and inorganic pollutants that might be present. The experiments with sand were done by P. Pankaj and the results are given in his thesis required for the degree of master in science in chemical engineering at the New Jersey Institute of Technology (1992).

### 3.1.3. Organism

*Phanerochaete chrysosporium* BKM-1767 (ATCC 24725) was obtained from the American Type Culture Collection (ATCC), Maryland. The culture was maintained on yeast malt extract agar medium. Yeast malt extract agar medium was prepared by desolving 0.3 g of yeast extract, 0.3 g of malt extract, 0.5 g of peptone, 2 g of agar and 1 g of glucose in 100 g of distilled water prior to autoclaving. After autoclaving, the medium was cooled to 50-60$^{o}$C and introduced in petri dishes.

### 3.1.4. Culture Media

All media and solutions were prepared using distilled water.

#### 3.1.4.1. Growth Medium

The fungus was grown separately from the soil and then added to the soil as it is described in Section 3.3.1.. The growth medium used to grow *Phanerochaete chrysosporium* is a modification of the recipe described by T. K. Kirk (Kirk et al., 1986) by P. Nirupam, PhD Student in chemical engineering at New Jersey Institute of Technology, Newark, NJ. The growth medium had the following composition:

| | |
|---|---|
| $KH_2PO_4$ | 2.00 g |
| $MgSO_4$ | 0.50 g |
| $CaCl_2$ | 0.10 g |
| $NaNO_3$ | 0.18 g |
| $NH_4Cl$ | 0.02 g |
| Glucose | 10.00 g |
| Thiamine | 1.00 mg |
| Mineral Salt Solution | 5.00 ml |

| | |
|---|---|
| Water | balance up to 1.00 l |

### 3.1.4.2. Induction Medium

The induction medium was used for Experimental Set # 1 (See Section 3.4.1). This medium was added to the soil prior to the addition of the fungal biomass, grown separately using the growth medium (as explained below in greater detail). The composition of the induction medium was a modification of the recipe of T. K. Kirk (Kirk et al., 1986) by N. Pal. It had the following composition 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 balance up to | 1.00 l |

The induction medium had a low concentration in glucose and nitrogen in order to stimulate enzyme production.

### 3.1.4.3. 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 lignolytic enzyme system. The composition of the mineral salt solution (Kirk et al., 1986) 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 g |
| $AlK(SO_4)_2,24H_2O$ | 10.00 mg |
| $H_3BO_3$ | 10.00 mg |
| $NaMoO_4$ | 10.00 mg |
| Nitriotriacetate | 1.50 g |
| Water | balance up to 1.00 liter |

5 ml of mineral salt water was added to each liter of growth and induction medium.

### 3.1.5. PCBs

Three different kinds of PCBs were used:

- Aroclor 1242
- Aroclor 1254
- Aroclor 1260

Aroclor 1242 was provided by Monsanto Company (St Louis, MO). Aroclor 1254 and 1260 was obtained from Chem Service Inc (West Chester, PA).

## 3.2. APPARATUS

### 3.2.1. Description of the Bottles Used

All the experiments with the fungus, PCBs and TCP, as well as the extraction tests and the extractions for Experimental Sets # 1, # 2 and # 3, were conducted with 20 g of soil that was introduced in bottles (as it is described in Section 3.3.2.). These bottles were Medium Round Bottles (clear glass) with Teflon-lined cap. The neck size was 58 mm and the inner volume was 250 ml. The Teflon-lined cap was used when the bottles were shaken during the extraction experiments (see Section 3.2.2.). During all the Experiments, the bottles were covered with cotton, but not capped, in order to allow air to enter the bottles.

### 3.2.2. Shakers

Two different types of shakers were used. One was used for the extractions and the other one was used for the fungal growth in Experimental Set # 1.

#### 3.2.2.1. Shaker Used for the Extractions

All the extractions performed during this work were conducted by shaking the bottles with their contents (the extraction procedures are described in section 3.3.4. for PCBs and 3.3.6. for TCP). The Teflon-lined caps were used to avoid leakage of the extraction solvent. The shaker (from Eberbach Corporation, Ann Arbor, Michigan) was shaking unidirectionally at 240 revolutions per minute.

### 3.2.2.2. Shaker Used for the Fungal Growth in Experimental Set # 1

As it is described in Section 3.3.1., for Experimental Set # 1, the fungus was grown in an erlenmeyer flask that was shaken to increase the oxygenation of the growth medium. The erlenmeyer was shaken bidirectionally with a Gyratory Water Bath Shaker provided by New Brunswick Scientific Co, Inc, (New Brunswick, N.J.) at 150 RPM.

### 3.2.3. Fermenter

In Experimental Sets # 2 and # 3, a significant amount of fungal inoculum was necessary. Therefore, the fungus was not be grown in an erlenmeyer flask. Instead, a fermenter was used to grow the fungus as described in Section 3.3.1.. The fermenter was a Bioflo II Batch/Continuous Fermenter (New Brunswick Scientific Co, Inc. Edison, NJ), that could contain 3 liter of fungal inoculum. Agitation, temperature and air flow rate were monitored and adjusted to set up the ideal conditions for fungal growth (as described in Section 3.3.1.)

### 3.2.4. Environmental Chambers

Environmental chambers were used to maintain a constant temperature and a constant humidity (as described in Section 3.4.) during the experiments. Two different environmental chambers were used. The first was used for Experimental Sets # 1 and # 2 and the second was used for Experimental Set # 3.

For Experimental Sets # 1 and # 2, a walk-in environmental chamber was used (Environmental Growth Chamber, Chagrin Falls, Ohio). The temperature was 30°C and the humidity 14%. Since we wanted a 100 % humidity environment and it was not possible to achieve this level with the environmental chamber itself, a system was built within the environmental chamber itself to keep the bottles at 100 % humidity. A plastic cover fixed on a metal frame was placed on a plastic receptacle filled up with 3 cm of water. The evaporation of that water increased the humidity in this closed system. When a high humidity was obtained in the systems, the bottles were introduced for the experiments.

For Experimental Set # 3, the environmental chamber used was provided by Tabai Espec. Corp. (model EY 101). In this environmental chamber, the temperature was 30°C and the humidity 100%. However, to increase the oxygenation of the samples, a manifold was introduced in this environmental chamber. The manifold is described in the next section.

### 3.2.5. Description of the Manifold Used in Experimental Set # 3

In Experimental Set # 3, described in Section 3.4.3. a continuous air flow was introduced in the bottles.

The air inlet was dry compressed air. The air was filtered with a filter (pore sizes = 0.3 um) to reduce contamination risks. The air was then bubbled through a 250 ml glass bubbler filled up with water (to have a high humidity and thus, not to dry the soil samples). Following the bubbler, the air line was connected to plastic Y-connectors (O.D. = 1/4 inch) linked together to form a manifold with 64 air outlets. Each outlet was introduced in a bottle containing an experimental sample. Each bottle was covered with cotton, through which the air tube was inserted.

## 3.3. METHODS

### 3.3.1. Fungal Growth

The fungus was grown differently for Experimental Set #1 and for Experimental Sets # 2 and # 3.

In Experimental Set # 1, because of the small amount of fungus needed (1 ml per bottle, as described in Section 3.4.1.), the fungus was grown in an erlenmeyer flask. The growth medium was autoclaved in an autoclavable closed bottle for 20 minutes at 121°C. An empty erlenmeyer flask covered with cotton and aluminum foil was autoclaved under the same conditions. After autoclaving, when the temperature of growth medium had reached room temperature, the growth medium was introduced in the erlenmeyer flask. Then, a loop was heated to red to avoid contamination and cooled down to take the fungal inoculum from the petri dishes described in Section 3.1.3. The aluminum foil and the cotton were removed from the flask. The fungus was introduced in the erlenmeyer flask with the loop. The erlenmeyer flask was covered with the cotton. This flask was shaken with the shaker described in Section 3.2.2.2. for 3 to 4 days at 39°C. Nitrogen and glucose concentration were analyzed. The fungus was added to the soil in the bottles when nitrogen concentration was below 2 ppm and when the glucose concentration was below 4 g/l.

For Experimental Sets # 2 and # 3, the fungus was grown in the fermenter described in Section 3.2.3. Two liters of autoclaved growth medium were introduced in the fermenter. The fungal inoculum was taken with a loop from the petri dishes described in Section 3.1.3. and introduced in the fermenter. The agitation in the fermenter was 150 RPM, the temperature was 35°C, and the air flow

rate was 1 liter per minute. To ensure that the fungus was ready to be used for the experiments, two parameters were controlled, namely, glucose and nitrogen concentrations. Nitrogen was the main parameter. The fungus was added to the bottles only when the nitrogen concentration in the fermenter was below 2 ppm. When the nitrogen concentration reached 10 to 20 ppm, it was analyzed more often, i.e. about every two hours, to know when the nitrogen concentration would reached 2 ppm. A glucose concentration below 4 g/l was considered acceptable for the transfer of the fungus to the contaminated soil (see Section 3.2.3 and Section 3.2.6. for the contamination of the soil with PCBs and TCP). If the growth medium initially contained 8 g/l of glucose, 0.02 g/l of $NH_4Cl$ and 0.18 g/l of $NaNO_3$, the concentration of glucose and nitrogen were both below the set threshold values (2 ppm for nitrogen and 4 g/l for glucose) after 3 or 4 days.

### 3.3.2. Soil Preparation

Twenty grams of soil were introduced in the 250 ml bottles. The Teflon-lined caps were not used for the soil preparation. For the two first experimental sets (described in Section 3.3.1. and 3.3.2.), the bottles with 20 grams of soil were covered with cotton and aluminum foil. They were autoclaved for 20 minutes at 121°C with the cotton and aluminum foil cover. For Experimental Set # 3 (described in Section 3.3.3.), cotton was not used during the soil sterilization. Instead, the bottles containing 20 g of contaminated soil were autoclaved for 20 minutes at 121°C covered only by aluminum foil.

### 3.3.3. PCB Addition

In the experiments with PCBs, this procedure was followed to add the PCBs to the sterilized soil in the autoclaved bottles. A 200 ppm solution of PCBs was first prepared using hexane as a solvent. 10 ml of this solution were added to each sterile bottle of soil. The bottles remained open (i.e. without any sort of cover) for 24 hours under the hood in order to completely evaporate the solvent. Each bottle contained 0.2 grams of PCBs for 20 grams of soil (i.e. 100 mg of PCBs per kilogram of soil).

### 3.3.4. PCB Extraction

At the end of each experiment, the PCB content of the bottles was extracted from the soil in order to determine the residual PCB concentration. In additions, preliminary extraction experiments were conducted to determine the extraction efficiency. All the extractions were conducted in the same 250 ml bottles that were used for the experiments. All these experiments were conducted with 20 grams of soil contaminated with 100 ppm PCBs and different quantities of fungal inoculum, induction medium and water as described below. The solvent used for the PCB extraction was always ether (50 ml). The bottles were closed with Teflon lined-caps to avoid leakage and shaken. Samples were taken during a 24 hour period. Each sample consisted of 1 ml of the ether phase. After sampling, 1 ml of fresh ether was reintroduced in the bottle (in order to conduct the extraction always with 50 ml of ether).

Several preliminary extraction experiments were run. One of them, done in duplicate, was conducted immediately after the addition of 1 ml of fungal inoculum, 10 ml of induction medium and 20 ml of water (in addition to the 20 grams of soil). The extraction was conducted with 50 ml of ether.

Another extraction experiment, also done in duplicate, was conducted with 20 ml of fungal inoculum added to 20 grams of soil. 50 ml of ether were used for the extraction.

In one instance, we tried to improve the extraction efficiency by adding NaCl. The experiment consisted in adding 4 grams of NaCl to 20 g of soil, 20 ml of biomass and 50 ml ether. A control extraction experiment was conducted without NaCl.

All the results for these extraction experiments are presented in Section 4.1.1.

### 3.3.5. PCB Analysis

The analyses were made on the ether phase using a gas chromatograph (GC) with a Electron Capture Detector (ECD).

Two different GCs were used. The first was used for Experimental Sets # 1 and # 2 (described in Section 3.3.1. and 3.3.2.), the second was used for Experimental Set # 3 (described in Section 3.3.3.)

**For Experiments Sets # 1 and 2:**

The analyses of these experiments were conducted by M. Shanon at Envirogen (Laurenceville, NJ)

Chromatograph: Varian

System ID 3400-8533

Gas: ECD-Carrier He-UHP 60 psi at regulator

Flow Rate: 0.67 ml/mn

Make-up gas-N2-UHP 60 psi at regulator

Flow Rate: 33 ml/mn

Split ratio: 16

Program: Initial column temperature: 160°C

2°C per minute for 20 minutes

8°C per minute for 5 minutes

240°C for 20 minutes

Injection temperature: 300°C

Detector temperature: 300°C

Volume injected: 1ul

Column used : DBI, Methylsilicone, 0.25 um film thickness. 30m; J & W Scientific (Varian Cat. N° JW 122103-20)

**For Experimental Set # 3**

Chromatograph: Hewlett Packard (HP)

5890 gas chromatograph

Gases: Carrier Gas : Helium 50 psi

Make up Gas : Nitrogen 30 psi

Flow rates: Split : 10.4 ml/mn

Purge : 3.2 ml/mn

Carrier gas (He) : 0.64 ml/mn

Make up + Carrier gas : 35.2 ml/mn

Program: Initial column temperature : 160 $^{O}$C

8 $^{O}$C / minute from 160$^{O}$C to 200$^{O}$C

1 $^{O}$C / minute from 200$^{O}$C to 240$^{O}$C

240 $^{O}$C / minute for 2 minutes

Injection temperature : 290 $^{O}$C

Detector: $^{63}$Ni ECD

Detector temperature: 290$^{O}$C

Column used: SPB™-608

Fused Silica Capillary Column 30m, 0.25 mm ID 0.25 um film thickness. SUPELCO, Inc.

An example of calibration curve is shown in Table 2 and Figure 3.

### 3.3.6. TCP Addition

TCP was added to some bottles (not containing PCBs) in the same way the PCBs were added. 10 ml of a 200 ppm solution of TCP in hexane was added to these bottles. The bottles remained open 24 hours under the hood in order to evaporate the solvent. When the solvent had evaporated, 100 mg of TCP per kilogram of soil remained in each of these bottles.

### 3.3.7. TCP Extraction

Some extraction experiments were conduct to determine what was the extraction efficiency of TCP. For these extraction experiments, TCP was added the same way it was added to the soil in the experiments. First, some preliminary tests were conducted (see Section 3.3.7.1.) to determine what extraction solvent to use. Then, four more extraction experiments (see Section 3.3.7.2.) were conducted to improve the procedure with the solvent that had been chosen (i.e. methanol). Finally, the adopted procedure was repeated four times to see if it was reproducible.

#### 3.3.7.1 Preliminary Tests of TCP Extraction Using Different Solvents

The Preliminary Extraction Tests were conducted to determine the extraction recovery efficiency of TCP from soil sample containing known amounts of TCP. These preliminary tests are described in Table 3.

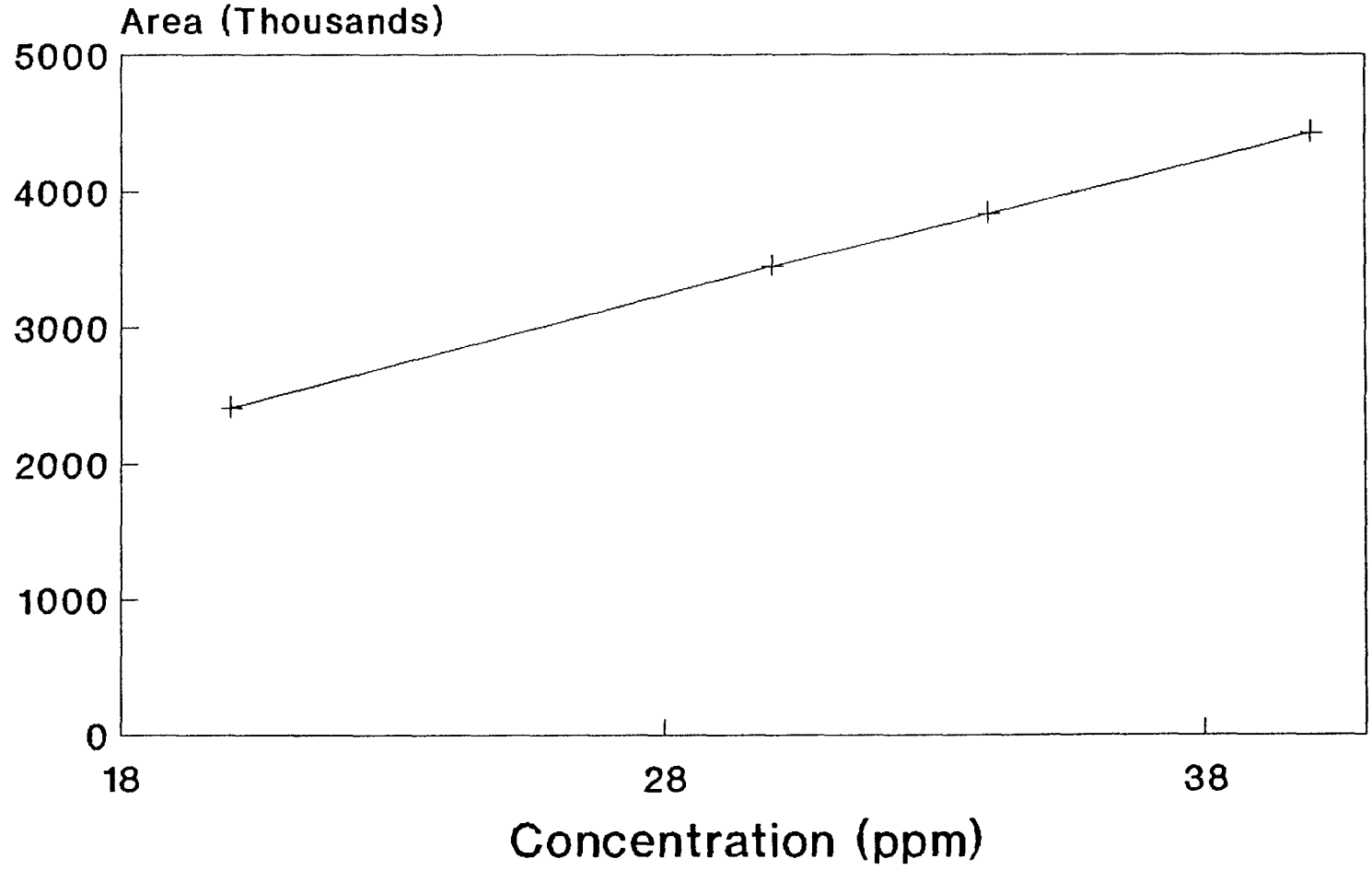
In Preliminary Test A1, 50 ml of water were added to the soil containing 100 ppm of TCP. A sample of 1 ml was taken after 3 hours of extraction and analyzed. In Test A2, 0.5g of KOH were added to the bottle used in Test A1 and shaken for 12 additional hours. The purpose of adding KOH was to increase the pH to solubilize more TCP (TCP is more soluble at high pH than it is at neutral pH). Test B1 consisted in the addition of 50 ml of biomass slurry to the soil. A sample of 1 ml was taken after 3 hours of extraction and analyzed. In Test B2, 0.5 g of KOH were added to the bottle used during Test B1 and the bottle was shaken for 12 additional hours. In Preliminary Test C, 20 ml of biomass, 100 ml of water and 1 g of KOH were added to the soil. One sample was taken for analyze after 2 hours and another one was taken 20 hours later (i.e. after 22 hours of total extraction time).

## Table 2 Calibration Curve for Aroclor 1254 Using the HP GC

| Concentration (ppm) | Area |
|---|---|
| 20 | 2,403,917 |
| 30 | 3,442,744 |
| 34 | 3,833,130 |
| 40 | 4,421,395 |

Correlation coefficient : 0.9998
Concentration = 9.01E-6 area - 3.9

# Figure 3
# GC Calibration Curve for Aroclor 1254




Correlation coefficient: 0.9998
Concentration = 9.01E-6 (area) - 3.9

## Table 3 Preliminary Tests of TCP Extraction Using Different Solvents

| Preliminary Test I.D. | Solvents (Added to 20 g of Soil) | Total Extraction Time (Hours) |
|---|---|---|
| A1 | Water (50 ml) | 3 |
| A2 | A1 + 0.5 g of KOH | 15 |
| B1 | Biomass (50 ml) | 3 |
| B2 | A2 + 0.5 g KOH | 15 |
| C | Biomass (20 ml)<br>Water (100 ml)<br>KOH (1 g) | 2<br>22 |
| D | Biomass (20 ml)<br>$CH_2Cl_2$ (50 ml)<br>Acetone (50 ml) | 2<br>22 |
| E | Biomass (20 ml)<br>Water (20 ml)<br>Methanol (50 ml) | 2.5 |
| F | Biomass (20 ml)<br>Water (20 ml)<br>Ether (50 ml) | 2.5 |

In preliminary test D, 20 ml of biomass, 50 ml of $CH_2Cl_2$ and 50 ml of acetone were added to the soil. Samples were taken for analysis after 2 and 22 hours of extraction. Preliminary test E was done with 20 ml of biomass, 20 ml of water and 50 ml of methanol added to the soil. A sample was taken for analyze after 2.5 hours of extraction. Preliminary test F was done with 20 ml of biomass, 20 ml of water and 50 ml of ether added to the soil. The ether phase was analyzed.

### 3.3.7.2. Experiment to Define the Optimal Extraction Process Using Methanol as a Solvent

From the results of the Preliminary Tests, methanol was chosen as extraction solvent as explained in the Results section. However, the procedure had to be improved. Therefore, four different methods were tried. They are described in Table 4. They were all conducted on 20 g of soil and 20 ml of biomass in the 250 ml bottles. The four methods consisted in three consecutive extractions with methanol. Different extraction times and different volumes of methanol were used.

Method M1: 30 ml of methanol were added to the test bottle containing the contaminated soil and the biomass. The extraction time, during which the bottles were shaken, was 1 hour. After this extraction, the soil was allowed to settle for two hours. The methanol phase was removed and a second aliquot of 30 ml of fresh methanol was added. The test bottle was extracted for 1 hour and then, the soil was allowed to settle for two hours. The methanol phase was removed and 20 ml of fresh methanol were added to the bottle. The extraction time was 1 hour. The solution was centrifuged and the liquid phase was removed. The three removed phases were added together in a 100 ml volumetric flask. The volume was balanced up to 100 ml with methanol. This solution was analyzed.

**Table 4 - Experiments to Define the Optimal Extraction Process for TCP Using Methanol as a Solvent**

| Method I.D. | Volume of the First Methanol Addition (ml) | First Extraction Time (hours) | Volume of the Second Methanol Addition (ml) | Second Extraction Time (hours) | Volume of the Third Methanol Addition (ml) | Third Extraction Time (hours) |
|---|---|---|---|---|---|---|
| M1 | 30 | 1 | 30 | 1 | 20 | 1 |
| M2 | 30 | 1/2 | 30 | 1/2 | 20 | 1/2 |
| M3 | 40 | 1/2 | 40 | 1/2 | 20 | 1/2 |
| M4 | 40 | 1/2 | 40 | 1/2 | 10 | 1/2 |

Methanol phase is removed and some fresh methanol is added  Methanol phase is removed

After the 2 first extractions, there was a 2 hours waiting period to allow the soil time to sediment. After the last extraction, a centrifugation replacesd the 2 hours waiting period. All the removed phases were introduced in a 100 ml volimetric flask. The level was balanced up to 100 ml with methanol and this solution was analyzed.

Method M2: This method is identical to method M1 but the three extraction times were 1/2 hour instead of 1 hour.

Method M3: The three extraction times were half an hour. The first and second methanol additions were 40 ml. 20 ml of methanol were added for the third solvent addition.

Method M4: The three extraction times were half an hour. 40 ml of methanol were used for the first and second additions of methanol. 10 ml methanol were used for the third addition of methanol.

Method M2 eventually was chosen as a standard extraction procedure for the experiments. However, this procedure was repeated four times to see if it was reproducible.

### 3.3.8. TCP Analysis

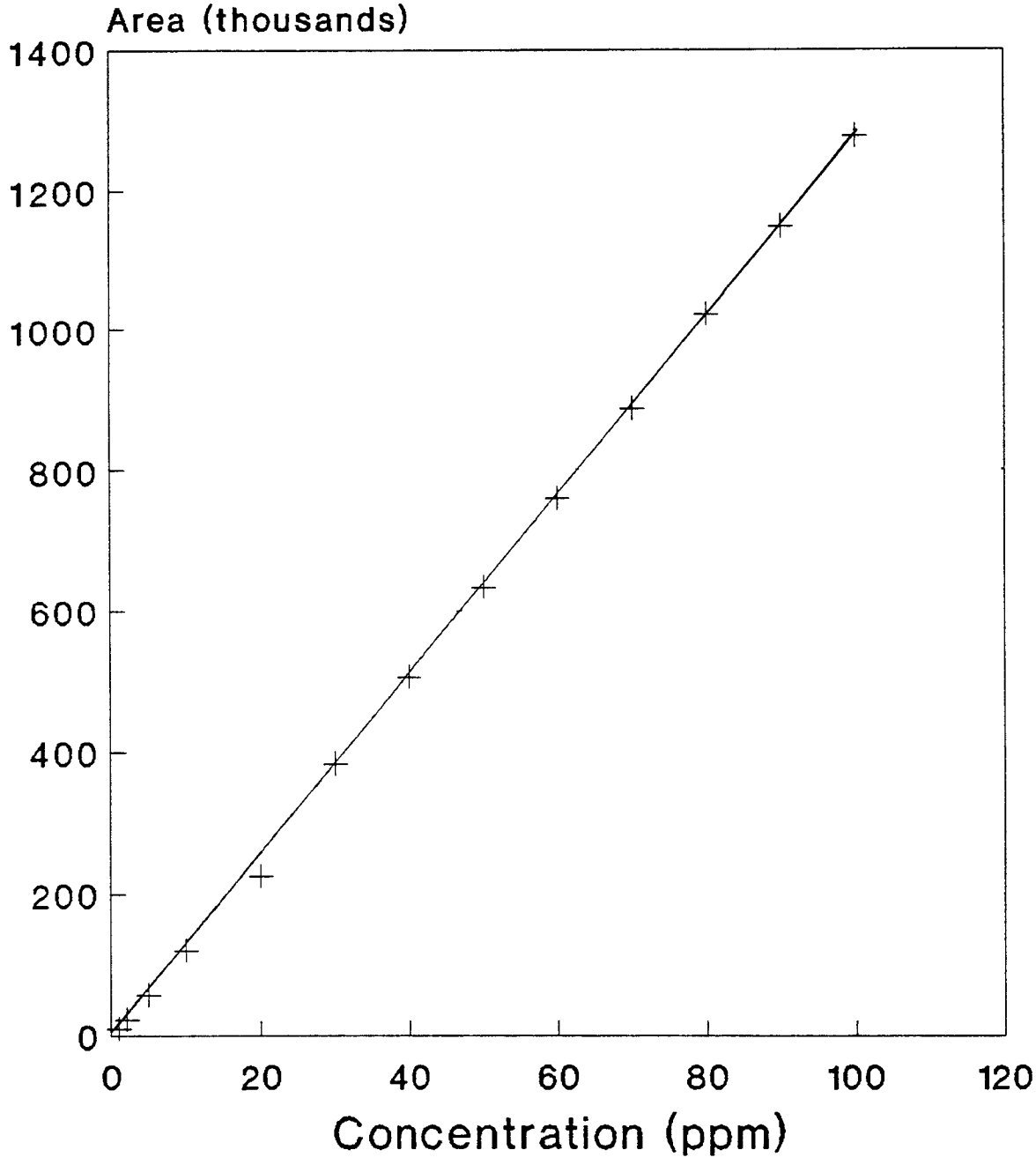
A Waters High-Performence Liquid Chromatography System was used with a Waters 600 E System Controller, Waters 715 Ultra Wisp Sample Processor, Water Tunable Absorbance Detector (at 280 nm). The column was Alltech Econosphere C8, 4.6 mm ID, * 150 mm (cat # 70090). An Alltech Direct Connect Refillable Guard Column is also used to protect the column. The mobile phase was methanol-water (60-40 by volume). The mobile phase flow rate was 1 ml/mn and the volume injected was 25 ul.

An example of calibration curve is given in Table 6 and Figure 4.

**Table 5** Calibration Curve for TCP using the HPLC.

| Concentration (ppm) | Area |
|---|---|
| 1 | 9.412 |
| 2 | 20,910 |
| 5 | 57,174 |
| 10 | 119,224 |
| 20 | 224,634 |
| 30 | 383,928 |
| 40 | 506,213 |
| 50 | 633,583 |
| 60 | 760,277 |
| 70 | 886,562 |
| 80 | 1,021,412 |
| 90 | 1,148,334 |
| 100 | 1,275,771 |

# Figure 4
# HPLC Calibration Curve for TCP



### 3.3.9. Addition of P. chrysosporium

The fungus was grown for 4 days in growth medium under the conditions described in Section 3.3.1., and was then directly added to each bottle. Nitrogen concentration, glucose concentration, and pH were tested before the addition of the fungus to the bottles. Nitrogen, the key parameter for the enzyme activity, had to be below 2 ppm and glucose below 4 g/l. However, in Experimental Set # 1, some additional nutrients (glucose and nitrogen) were added to find the best conditions for the biodegradation (see 3.3. Conditions for the Experiments).

## 3.4. Experimental Conditions

The experiments consisted in exposing PCB- or TCP- contaminated soil to the fungus. All the experiments were conducted with 20 g of 100 ppm (PCB or TCP) contaminated soil. The soil was introduced in the 250 ml bottles, autoclaved and contaminated as described in Section 3.3.2.. Depending on the experimental set, different quantities of fungal inoculum and induction medium were added. Other parameters such as glucose concentration, nitrogen concentration and the oxygenation rate of the soil sample were also modified from an experimental set to another. However, all the experimental sets were conducted at 30°C, and the air surrounding the bottles had 100% humidity to avoid evaporation. To stop the experiments at a given time, the bottles were placed in a freezer (-10°C) to block any biological activity.

Furthermore, for each experimental set, a number of bottles (all identically prepared) were set up. The contents of these identical bottles were analyzed at different times (one bottle at a time) in order to follow the time course of the

experiment. When an analysis was performed, the content of the designated bottle was sacrificed.

### 3.4.1. Experimental Set # 1 with 1 ml of Fungal Inoculum Added to PCB-Contaminated Soil

These experiments were attempted with EPA soil contaminated with 100 ppm of Aroclor 1254. The purpose was to determine if PCBs were biodegraded and which were the best conditions for this biodegradation. Two experiments were also attempted with EPA soil contaminated with 100 ppm of Aroclor 1242 and 1260 in order to compare the results for the three different types of PCBs.

Three parameters were studied:

- Glucose Concentration (10 and 20 g/kg of soil)
- Nitrogen Concentration (0 and 32 mg per kg of soil)
- Water Content (35 and 48 % by mass)

Control bottles were also prepared. These bottles contained no fungus but only either autoclaved or non-autoclaved soil. The experimental conditions with Aroclor 1254 are described in Table 6 and Table 7. The experiment conditions with Aroclor 1260 and 1242 are described in Table 8. Two sets of controls were conducted without fungus.

For the reason described above, the same conditions were repeated in five different bottles.

Table 6 Experimental Conditions for Runs in Experimental Set # 1 with Aroclor 1254 in Soil and 1ml of Fungal Inoculum

| Initial Water Content (%) | Initial $NH_4Cl$ Concentration (ppm) | Initial Glucose Concentration (g/l) | Number of Bottles |
|---|---|---|---|
| 35 | 0 | 10 | 5 |
| 35 | 32 | 20 | 5 |
| 35 | 0 | 10 | 5 |
| 35 | 32 | 20 | 5 |
| 48 | 0 | 10 | 5 |
| 48 | 32 | 20 | 5 |
| 48 | 0 | 10 | 5 |
| 48 | 32 | 20 | 5 |

Table 7 Experimental Conditions for Controls in Experimental Set # 1 (Aroclor 1254 in Soil)

| Initial Water Content (%) | Soil | Initial $NH_4Cl$ Concentration (ppm) | Initial Glucose Concentration (g / l) | Number of Bottles |
|---|---|---|---|---|
| 48 | non sterile | 0 | 0 | 5 |
| 48 | non sterile | 32 | 10 | 5 |
| 48 | sterile | 0 | 0 | 5 |
| 48 | sterile | 32 | 10 | 5 |

Table 8 Experimental Conditions for Runs in Experiment # 1 with Aroclor 1242 and 1260 (in Soil with 1 ml of Fungal Inoculum)

| Initial Water Contain (%) | Initial NH4Cl Concentration (ppm) | Initial Glucose Concentration (g / l) | Number of Bottles |
|---|---|---|---|
| 48 | 32 | 10 | 5 |

There was one set of experiments for Aroclor 1242 and one set for Aroclor 1260. There was also one set of control experiments for each Aroclor (in which 1 ml of fungal inoculum was replaced by 1 ml of induction medium)

### 3.4.2. Experimental Set # 2 with 15 ml Inoculum Added to EPA Soil

This experimental set was conducted on 20 g of EPA soil contaminated with 100 ppm of Aroclor 1254. 15 ml of independently grown fungus (see Section 3.2.1. Fungal Growth) was added to the soil sample in order to determine the degradation effectiveness on PCBs in a system where the amount of fungal biomass and proteins was significant. The fungal biomass had a nitrogen concentration below 2 ppm and a glucose concentration below 4 g/l.

### 3.4.3. Experimental Set # 3 with PCB-Contaminated Soil and TCP-Contaminated Soil

This experiment was carried with 20 g of 100 ppm PCB contaminated soil and 20 ml of fungal biomass. A set of control experiments with TCP-contaminated soil was set up in parallel. These control experiments were carried out to insure that the fungus was active. Therefore, the experiments with PCBs and TCP were conducted at the same time. The fungus came from the same original batch.

An air flow was continuously introduced into the bottles. The manifold described in Section 3.2.5. was used to introduce this additional air.

To follow the time course of the experiment, 5 bottles with PCB-contaminated soil were set up in identical initial conditions. For the same reason, 6 bottles with TCP-contaminated soil were also set up in the identical initial conditions.

**Table 9 Experimental Conditions for Runs in Experimental Set # 3 with Aroclor 1254 and TCP in 20 g of Soil and 20 ml Fungal Inoculum**

| Quantity of Soil (g) | Chemical Contaminant | Contaminant Concentration (ppm) | Volume of Fungal Inoculum (ml) | Number of Bottles |
|---|---|---|---|---|
| 20 | PCBs | 100 | 20 | 5 |
| 20 | PCBs | 100 | 0 | 5 |
| 20 | TCP | 100 | 20 | 6 |
| 20 | TCP | 100 | 0 | 6 |

# Chapter 4 Results

## 4.1. Extraction Efficiency

### 4.1.1. PCB Extraction Efficiency

In order to determine the extraction efficiency of PCBs from a soil sample as a function of extraction time, test runs were conducted. In these runs, the solvent (ether) was added to a bottle containing 20 g of contaminated soil, 10 ml of induction medium and 1 ml of fungal inoculum. The bottle was shaken and samples were taken from the ether phase at periodic time intervals. The results are shown in Table 10 and Figure 5. The extraction gave 100 % recovery after a 10 hour extraction for Run # 1 and 100 % after 14 hours for Run # 2. Both runs were conducted under the same conditions. For some of the results, the recovery is above 100 % (up to 105 %). The high volatility of ether might be the explanation for this excess of PCB recovered. As samples were taken out of the extraction bottles, the bottles had to be opened. Each time a bottle was open, some ether would evaporate. The raw data in Table 10 were interpreted assuming that the rate of PCB extracted from the soil at any given time is proportional to the amount of PCB still in the soil. Mathematically, this implies that:

$$y = yo\ (1\text{-}e^{-Kt})$$

where y = PCB in the extraction fluid (in mg)

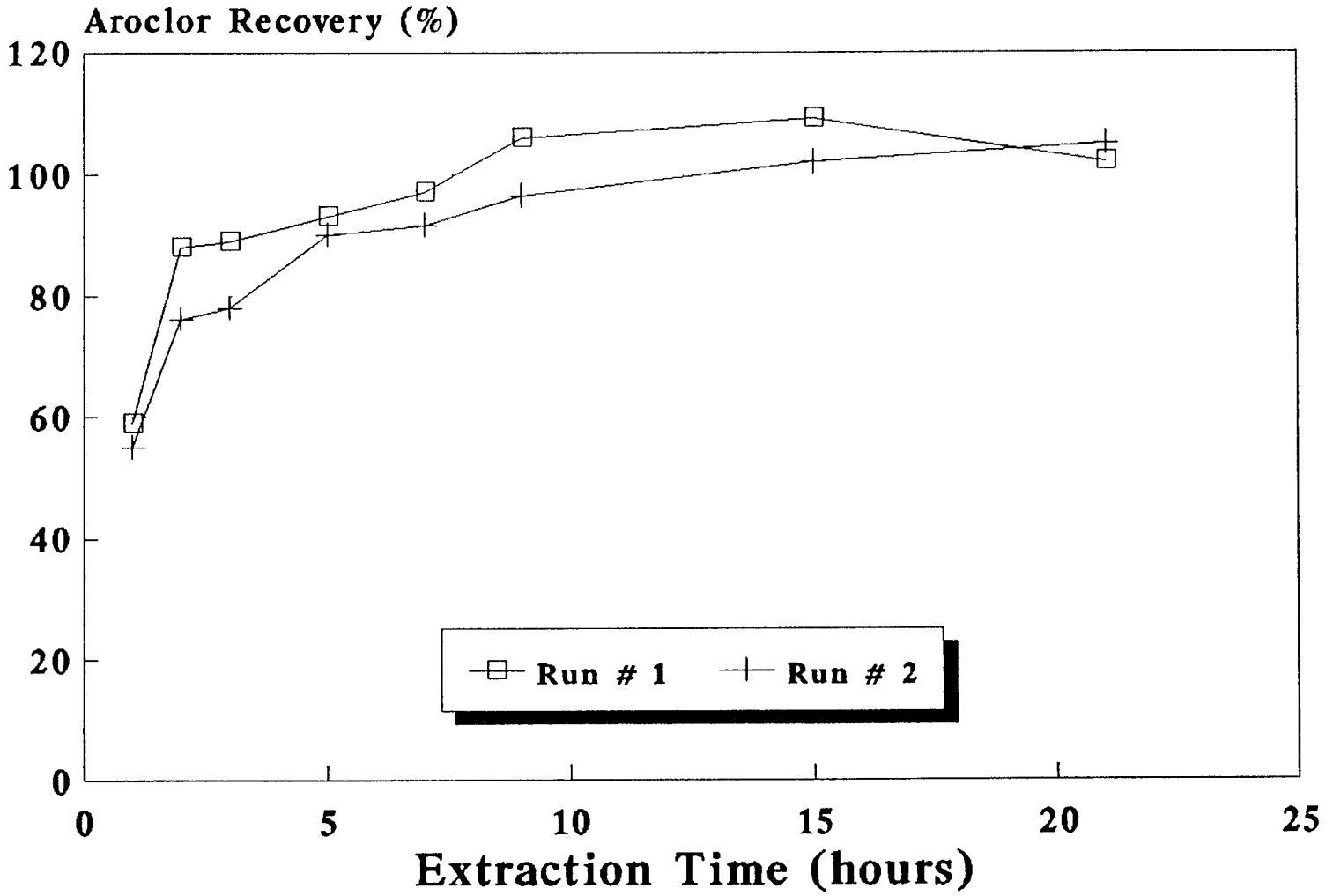
yo = PCB initially present in the soil (in mg)

## Table 10 - Aroclor 1254 Extraction with 1 ml Fungal Inoculum

(1ml fungal inoculum, 10ml induction medium, 20 g of 100 ppm contaminated soil, 50ml ether)

| Extraction Time (hours) | Run # 1 | | Run #2 | |
|---|---|---|---|---|
| | PCB (mg/bottle) | (% recovery) | PCB (mg/bottle) | % recovery |
| 1 | 1.18 | 59 | 1.10 | 55 |
| 2 | 1.76 | 88 | 1.53 | 76 |
| 3 | 1.79 | 89 | 1.56 | 78 |
| 5 | 1.86 | 93 | 1.80 | 90 |
| 7 | 1.94 | 97 | 1.83 | 91 |
| 9 | 2 12 | 106 | 1.93 | 96 |
| 15 | 2.18 | 109 | 2.04 | 102 |
| 21 | 2.04 | 102 | 2.10 | 105 |

Figure 5 Extraction Efficiency of Aroclor 1254 from Soil(with 1 ml of Fungal Inoculum and 10 ml of Induction Medium)as a Function of Time



This equation can be rewritten as

$$\ln[(yo-y)/yo] = -Kt$$

This equation was used to interpret the data reported in Table 10. The results are presented in Table 11 and Figure 6. The slope of the graph in this figure is the constant K. K was determined using only the two first extraction times (1 and 2 hours). Since, for long extraction times, the difference between $y_o$ and y is very small, this implies that a very small error in the analysis could result in a very significant error in the value of ln[(yo-y)/yo]. Therefore, it would not be accurate to use the data obtained for long extraction times. Under these approximations, the value of K was found to be equal to 0.85 $day^{-1}$

The corresponding data for the experiments in which 20 ml of fungal biomass were added to 20 g of contaminated soil are presented in Table 12 and Figure 7, 7a and 7b. In this experiment, recovery above 100 % was observed for Run # 1 (up to 102 %). However, for Run # 2 the recovery was not higher than 92 %. No real explanation can be given for this poor recovery. The value of K for Run # 1 was found to be equal, in this case, to 1.06 $day^{-1}$. The value of K for Run # 2 was found to be equal to 0.72 $day^{-1}$.

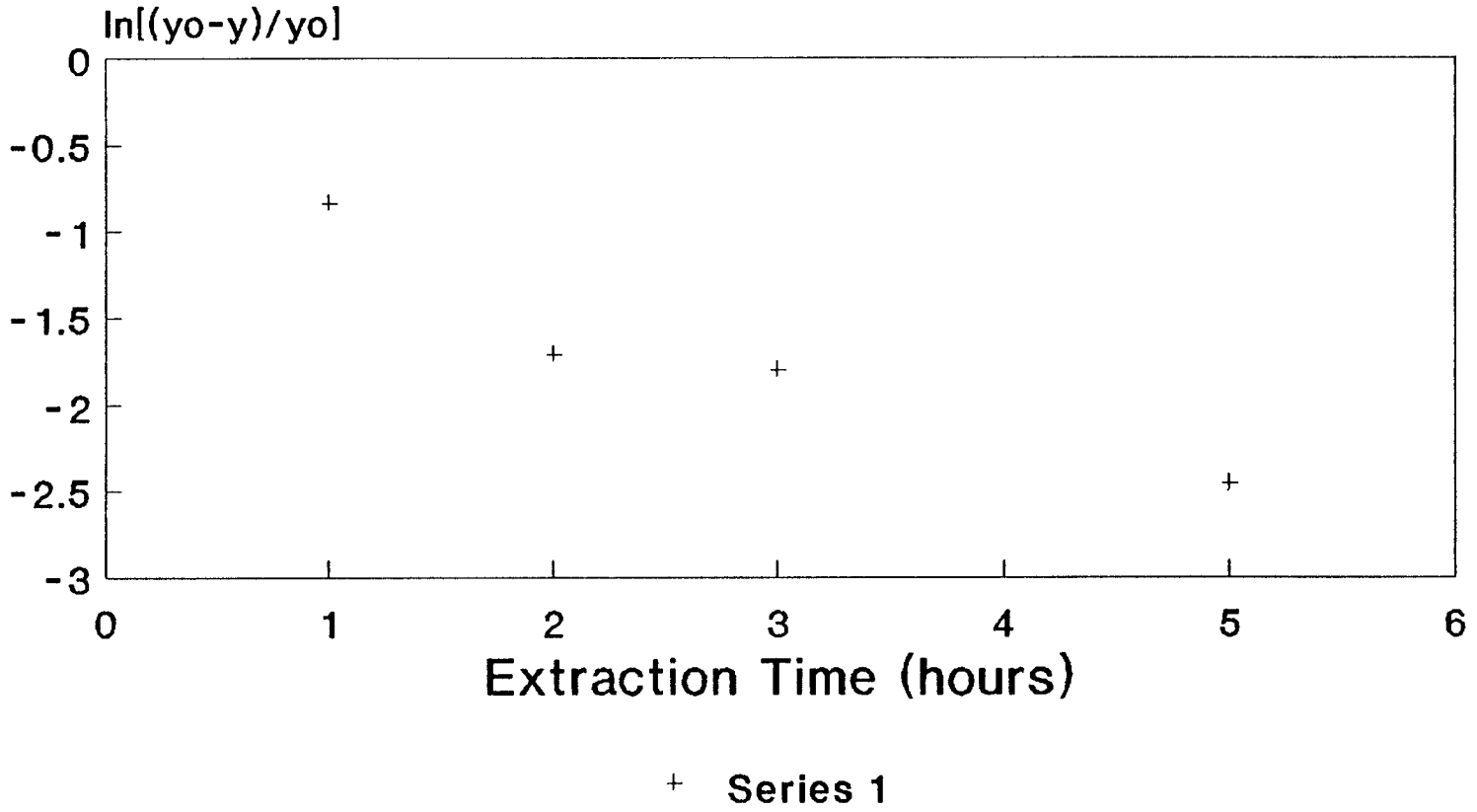
The data for the extraction experiments in which additions of NaCl were made are presented in Table 13 and Figure 8. The extraction with additional salt was faster than the control extraction. 100 % recovery was obtained in 5 hours with additional salt. The problem with NaCl was that after 5 hours, the recovery started to decrease and reach 5.3 % in 23.5 hours. The same kind of results were obtained with sand.

**Table 11 Determination of ln[(yo-y)/yo] from the Extraction Experiment with 1 ml of Fungal Inoculum, 10 ml of Induction Medium and 20 g of Soil**

| Extraction Time (hours) | y = Average PCB Extracted (mg) | (yo-y)/yo | ln [(yo-y)/yo] |
|---|---|---|---|
| 1 | 1.14 | 0.43 | -0.84 |
| 2 | 1.64 | 0.18 | -1.71 |
| 3 | 1.67 | 0.165 | -1.80 |
| 5 | 1.83 | 0.085 | -2.46 |

yo = PCB present in theSoil (2mg)
y is the Average of the PCB Extracted from Run # 1 and Run # 2 for the Same Extraction Time

Figure 6 ln[(yo-y)/yo] Function of the Extraction Time in 20 g of Soil, 1 ml Fungal Inoculum and 10 ml Induction Medium



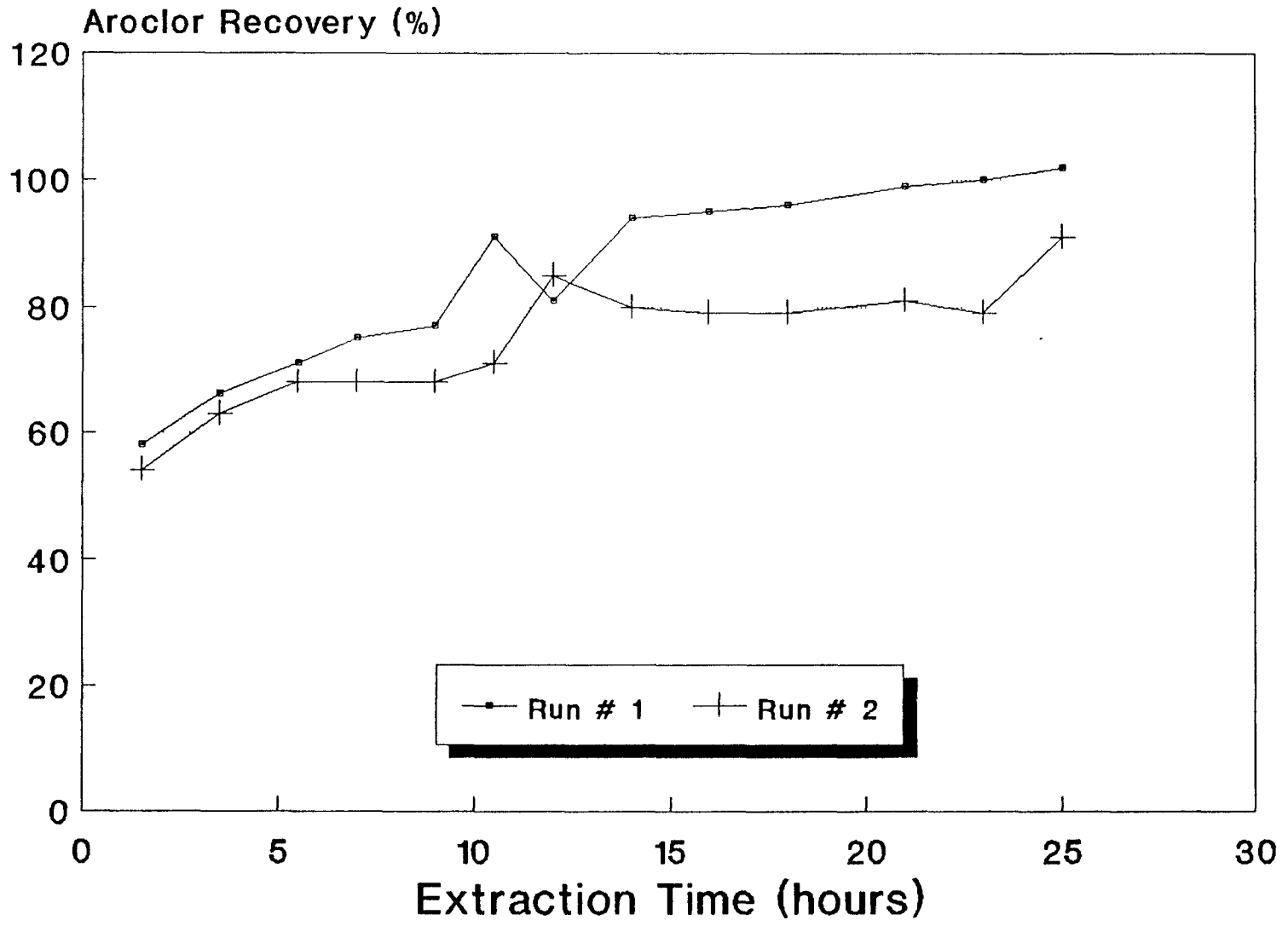

yo = PCB in Soil (mg)
y = PCB Extracted (mg)

## Table 12 - Aroclor 1254 Extraction with 20 ml Fungal Inoculum

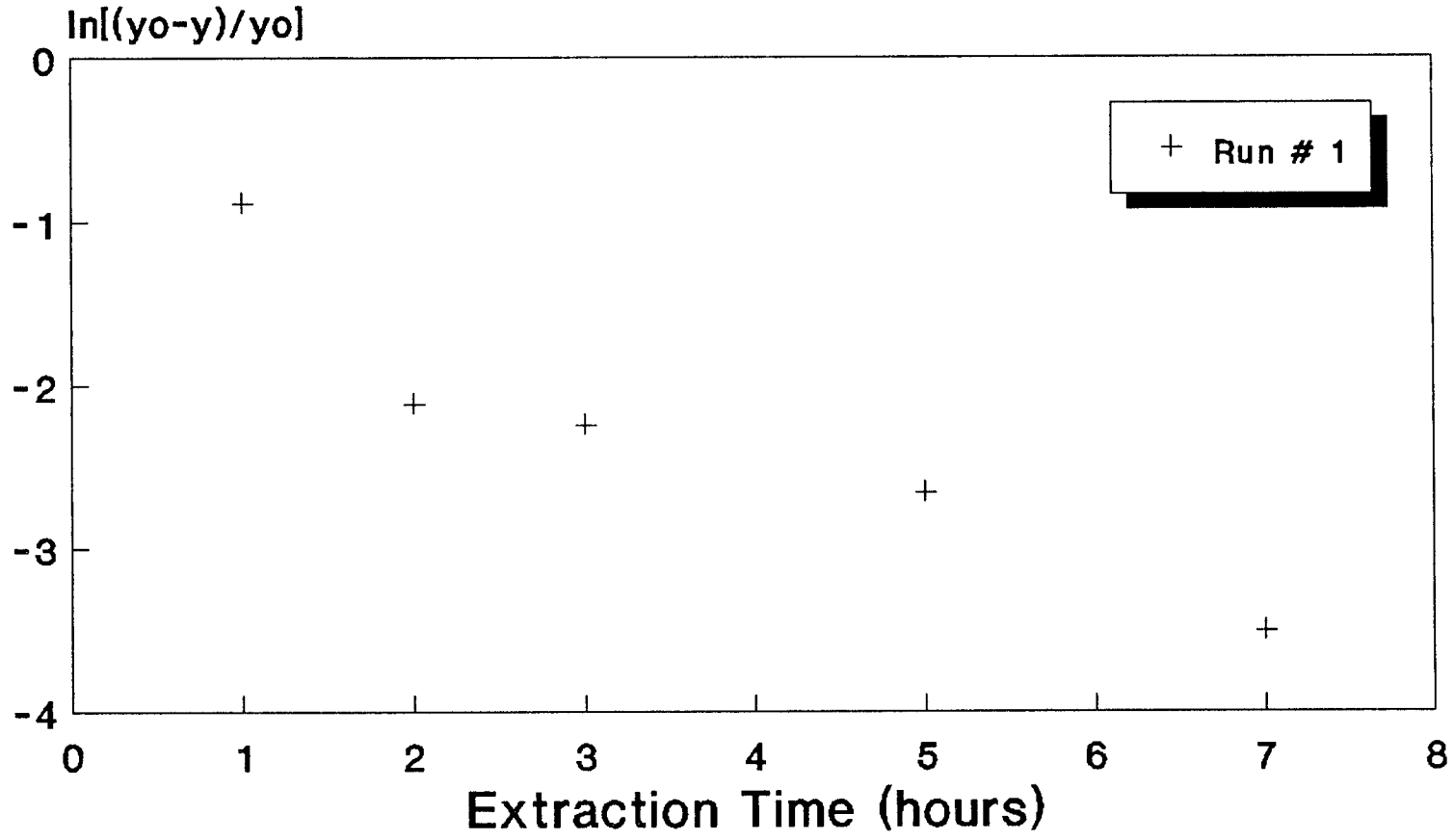
(20 ml fungal inoculum, 20g of 100 ppm contaminated soil, 50ml ether)

| Extraction Time (hours) | Run # 1 | | Run # 2 | |
|---|---|---|---|---|
| | PCB (mg/bottle) | % recovery | PCB (mg/bottle) | % recovery |
| 1.5 | 1.16 | 58 | 1.08 | 54 |
| 3.5 | 1.31 | 66 | 1.27 | 63 |
| 5.5 | 1.38 | 71 | 1.37 | 68 |
| 7 | 1.43 | 75 | 1.37 | 68 |
| 9 | 1.45 | 77 | 1.37 | 68 |
| 10.5 | 1.69 | 91 | 1.42 | 71 |
| 12 | 1.46 | 81 | 1.70 | 85 |
| 14 | 1.68 | 94 | 1.61 | 80 |
| 16 | 1.68 | 95 | 1.58 | 79 |
| 18 | 1.66 | 96 | 1.58 | 79 |
| 21 | 1.68 | 99 | 1.62 | 81 |
| 23 | 1.68 | 100 | 1.59 | 79 |
| 25 | 1 69 | 102 | 1.83 | 91 |

Figure 7 Extraction Efficiency of Aroclor 1254 with Soil (with 20 ml of Biomass) as a Function of Extraction Time

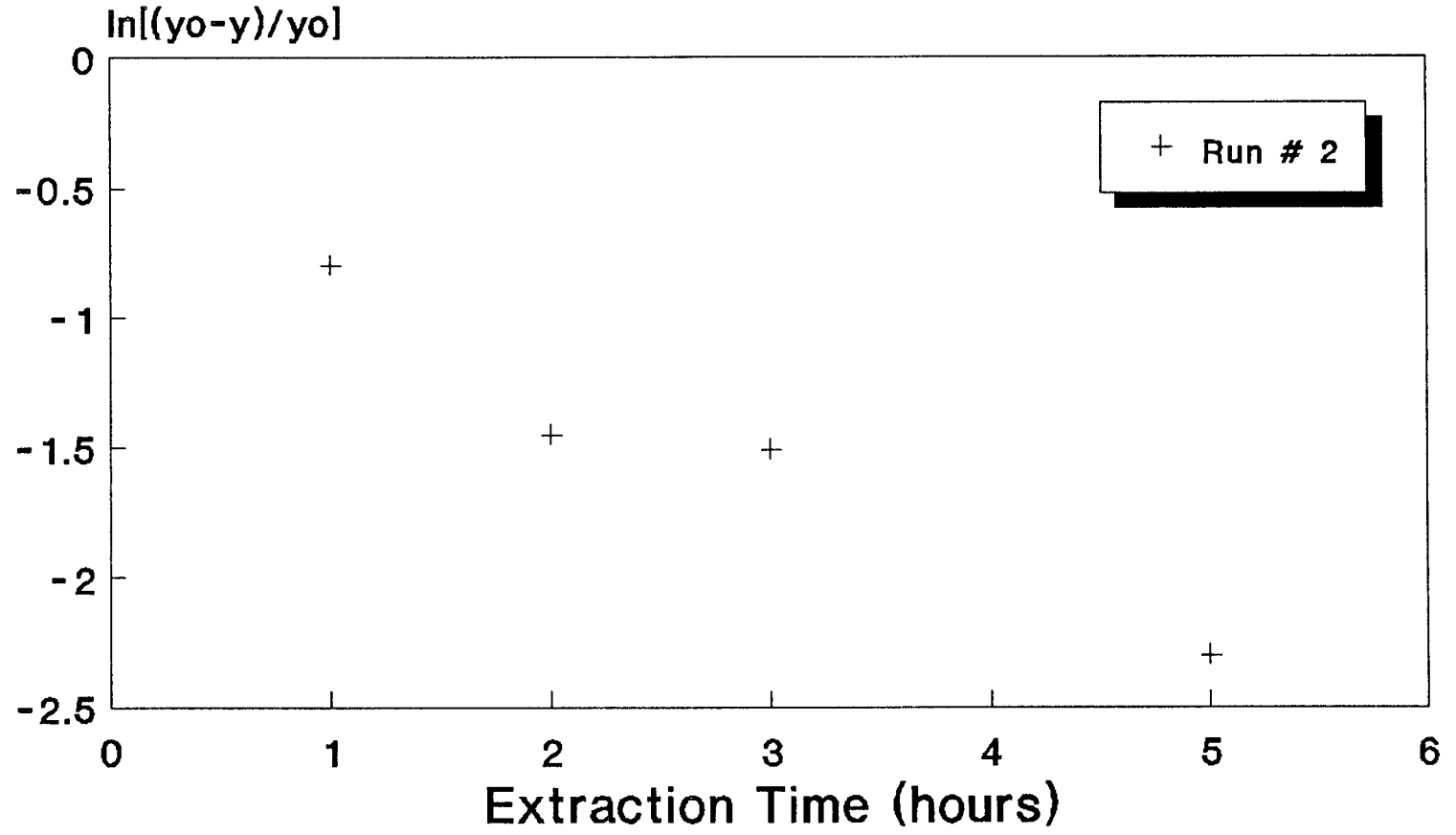


# Figure 7a ln[(yo-y)/yo] Function of the Extraction Time in 20 g of Soil and 20 ml Fungal Inoculum for Run # 1




yo = PCB in the extraction fluid (in mg)
y = PCB initially present (in mg)

# Figure 7b ln[yo-y)/yo] Function of the Extraction Time in 20 g of Soil and 20 ml Fungal Inoculum for Run # 2

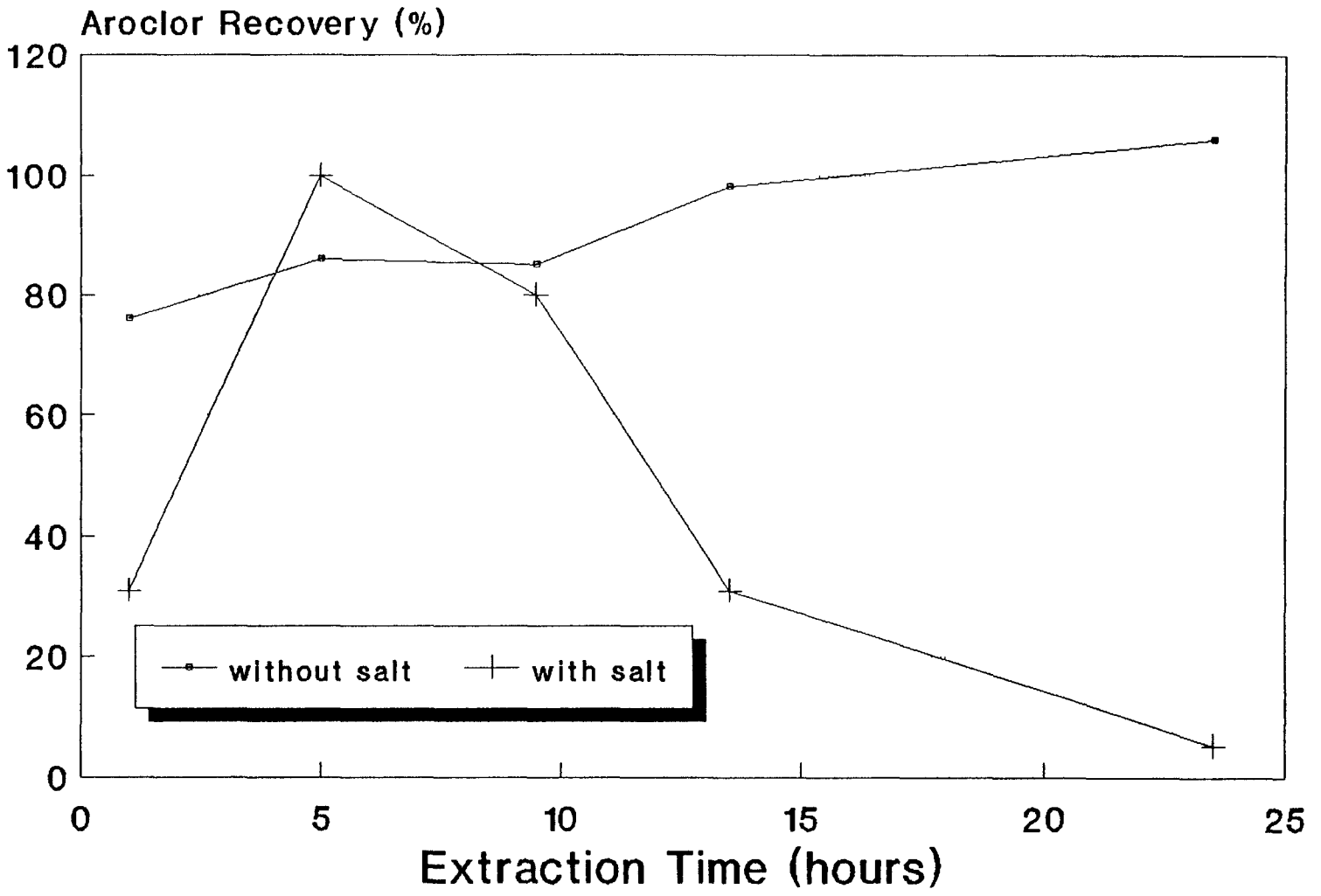



yo = PCB in the etraction fuid (in mg)
y = PCB iitially Present (in mg)

## Table 13 - Aroclor 1254 Extraction - Experiment in 100 ppm PCB Contaminated Soil with and without NaCl Addition

| Extraction Time (hours) | without NaCl | | with NaCl | |
|---|---|---|---|---|
| | PCB (mg/bottle) | % recovery | PCB (mg/bottle) | % recovery |
| 1 | 1.52 | 76 | 0.62 | 31 |
| 5 | 1.72 | 86 | 2.00 | 100 |
| 9.5 | 1.70 | 85 | 1.60 | 80 |
| 13.5 | 1.97 | 98 | 0.75 | 31 |
| 23.5 | 2.13 | 106 | 0.11 | 5.3 |

Figure 8 Extraction Efficiency of Aroclor 1254 from Soil with and without salt as a function of Extraction Time (with 20 ml of Fungal Inoculum)



Without any addition of salt, 100 % recovery was obtained in 15 hours which was about what was obtained in the former experiments under the same conditions. The recovery remained above 100 % until the end of the experiment. Therefore, the salt addition was not used any further. Instead, a 23 hour extraction with 50 ml ether without addition of salt was used for all the extractions in Experimental Set # 3.

Experimental Sets # 1 and # 2 were conducted before specific extraction tests were conducted. Different extraction times between 1.5 to 12 hours were used for these two sets of experiments.

### 4.1.2. TCP Extraction Efficiency

#### 4.1.2.1. Results from the Preliminary Tests Using Different Solvents

The results of these preliminary tests are presented in Table 14.

Preliminary Test A1 gave 0 % recovery. No TCP was extracted from the soil. Test B1 gave also very poor results (26 % recovery after 3 hours of extraction). Most of the TCP remained in the soil. At this point it was decided that a more effective procedure had to be found. As a first attempt, KOH was added to Preliminary Tests A1 and A2, and the extraction time was increased. The recovery was then much higher. 70 % recovery were obtained with PT A2 and 78 % were obtained with B2. This shows that for Preliminary Tests A1 and B1, TCP was present in the soil but was not extracted. A more basic pH and a higher extraction time was necessary to solubilize more TCP.

Preliminary Test C gave 61 % recovery after 2 hours and 82 % after 20 hours of extraction. Preliminary Test D gave 77 % recovery after 2 hours but the recovery decreased when the sample was shaken longer (73 % after 20 hours).

## Table 14 Preliminary Tests of TCP Extraction Using Different Solvents

| Preliminary Test I.D. | Solvents (Added to 20 g of Soil) | Total Extraction Time (Hours) | TCP Recovery (%) |
|---|---|---|---|
| A1 | Water (50 ml) | 3 | - |
| A2 | A1 + 0.5 g of KOH | 15 | 70 |
| B1 | Biomass (50 ml) | 3 | 26 |
| B2 | A2 + 0 5 g KOH | 15 | 78 |
| C | Biomass (20 ml)<br>Water (100 ml)<br>KOH (1 g) | 2<br>22 | 61<br>82 |
| D | Biomass (20 ml)<br>$CH_2Cl_2$ (50 ml)<br>Acetone (50 ml) | 2<br>22 | 77<br>73 |
| E | Biomass (20 ml)<br>Water (20 ml)<br>Methanol (50 ml) | 2.5 | 78 |
| F | Biomass (20 ml)<br>Water (20 ml)<br>Ether (50 ml) | 2.5 | 101 |

Preliminary Test E (with methanol) gave 78 % recovery after 2.5 hours extraction. With ether (Preliminary Test F), 101 % recovery was obtained in 2.5 hours.

The best results were obtained with ether. However, this solvent was not chosen for the experiments because of its volatility. The result would not have been reproducible enough when analyzing the samples with the HPLC. The 101 % recovery obtained with ether showed that, for the other experiments, the TCP missing was due to a bad extraction efficiency and not from a problem in the addition procedure.

Therefore, the methanol extraction (78 % recovery after 2.5 hours of extraction time) was chosen as a standard extraction method for all subsequent experiments.

#### 4.1.2.2. Results of the Experimental Run to Define the Optimal Extraction Process

From the Preliminary Tests, methanol was chosen as the extraction solvent. However, the procedure had to be improved. Therefore, four methanol extraction methods were conducted. These methods, described in Section 3.3.7., all resulted in higher extraction recovery (between 79 and 90 %). These results are presented in Table 15. Method M2 gave the highest extraction efficiency. Therefore, this procedure was chosen for the experiments.

## Table 15 - Experiments to Define the Optimal Extraction Process for TCP using Methanol as a Solvent

| Method I.D. | Volume of the First Methanol Addition (ml) | First Extraction Time (hours) | Volume of the Second Methanol Addition (ml) | Second Extraction Time (hours) | Volume of the Third Methanol Addition (ml) | Third Extraction Time (hours) | Cumulative TCP Recovery (%) |
|---|---|---|---|---|---|---|---|
| M1 | 30 | 1 | 30 | 1 | 20 | 1 | 84.5 |
| M2 | 30 | 1/2 | 30 | 1/2 | 20 | 1/2 | 90 |
| M3 | 40 | 1/2 | 40 | 1/2 | 20 | 1/2 | 83 |
| M4 | 40 | 1/2 | 40 | 1/2 | 10 | 1/2 | 79.5 |

Methanol phase is removed and some fresh methanol is added

Methanol phase is removed

After the 2 first extractions, there was 2 hours waiting to let the soil time to sediment. After the last extraction, a centrifugation replaced the 2 hours waiting. All the removed phases were introduced in a 100 ml volumetric flask. The volume was balanced up to 100 ml and the solution was analyzed..

Extraction Tests using Method M2 were conducted four more time to determine if the extraction process was reproducible or not. The following percentage recovery were obtained: 92 %, 95 %, 97 % and 98 %. This is an average of 95.5 % recovery (the 90 % first obtained were not considered in this average because the operator was not fully acquainted with the procedure). Table 16 fully describes the Adopted Extraction Procedure.

## 4.2. Results for Experimental Set # 1 and # 2

All the results obtained indicate that no degradation of PCBs is taking place in any of the experiments performed in this work. A typical series of results is presented in Figure 9 for Experimental Set # 1. This figure shows the chromatograms of the extracts from the bottles containing PCBs and inoculated with the fungus, as well as the extract from a control experiment. If any kind of biodegradation were occurring, one would expect to see a decrease in some of the peaks of the chromatograms. These peaks represent one or more different congeners of the aroclor. The figure shows that no such decrease occurs as time progresses. In fact, the control chromatogram appears to have the same exact fingerprint of the other chromatograms.

Any change in the relative heights of corresponding peaks in different chromatograms could be attributed to possible variations in the calibration of the GC or, more significantly, to the incompleteness of the extraction process used to recover the aroclor from the soil and biomass. For example, the partial effectiveness of the extraction is clearly shown with sand in Figure 10, which contains a plot of the Aroclor 1260 recovered in the extract versus the age of the culture.

## Table 16 Adopted TCP Extraction Procedure

The extraction is done from 20 g of 100 ppm contaminated soil and 20 ml of fungal inoculum.

| |
|---|
| First Addition of Methanol (30 ml) |
| First Extraction Time (1/2 hour) |
| Two Hour Waiting for the Soil to Sediment |
| Removal of the Methanol Phase |
| Second Addition of Methanol (30 ml) |
| Second Extraction Time (1/2 hour) |
| Two Hour Waiting for the Soil to Sediment |
| Removal of the Methanol Phase |
| Third Addition of Methanol (20 ml) |
| Third Extraction Time (1/2 hour) |
| Centrifugation |
| Removal of the Liquid Phase |

# Chromatograms of Aroclor 1254 Extracted from Soil

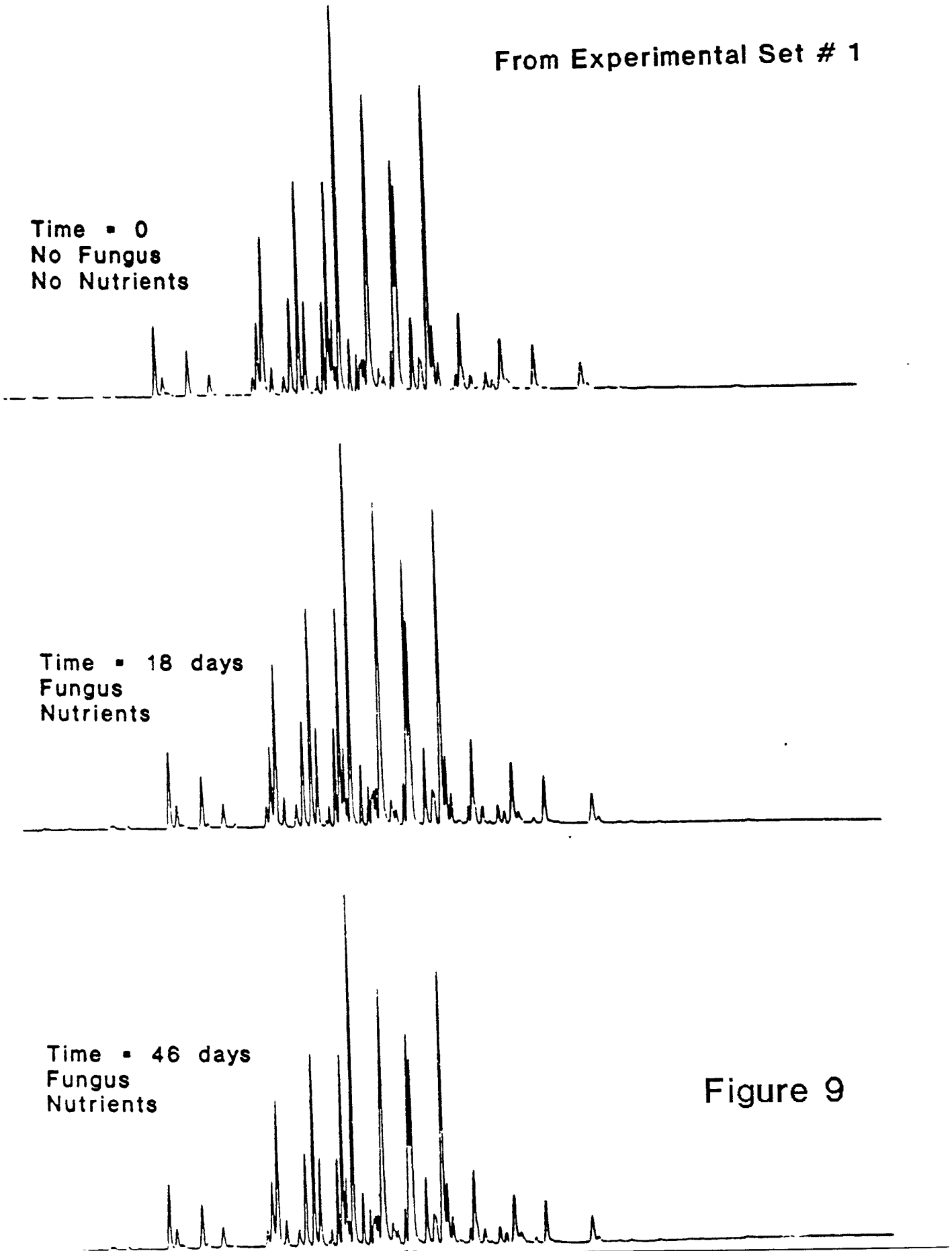
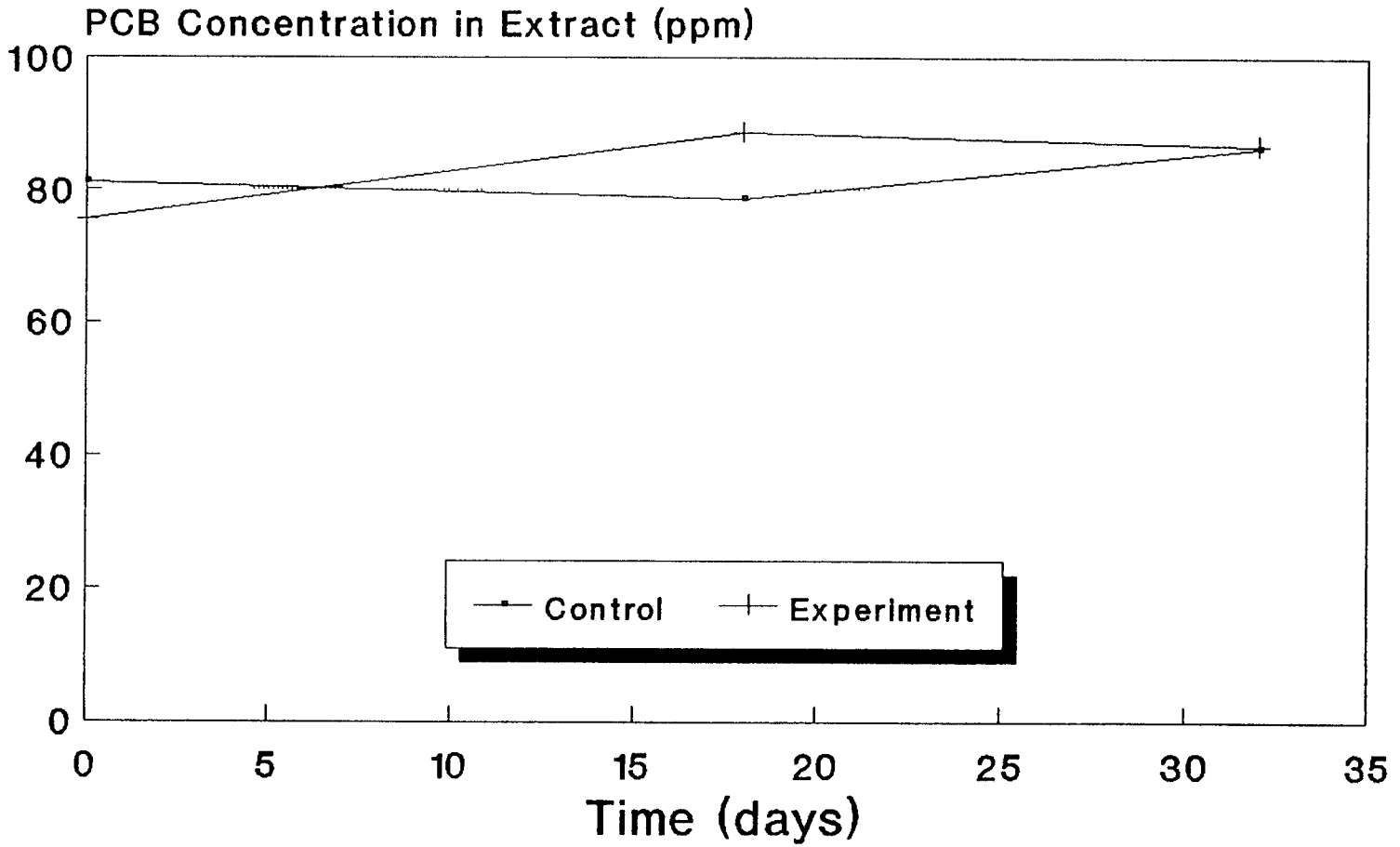



Figure 9

# Figure 10
# Fungal Biodegradation Effectiveness Against Aroclor 1260 in Sand




Experimental Set # 1 Initial Concentration in Sample = 100 ppm of PCBs

One can see that amount of PCB contained in the extracts from the control experiments and those for the actual experiments are not 100% of the amount of Aroclor initially added to the bottles.

The effectiveness of the extraction was obtained by analyzing the content of PCBs in the extract at different extraction times using the previously derived equation:

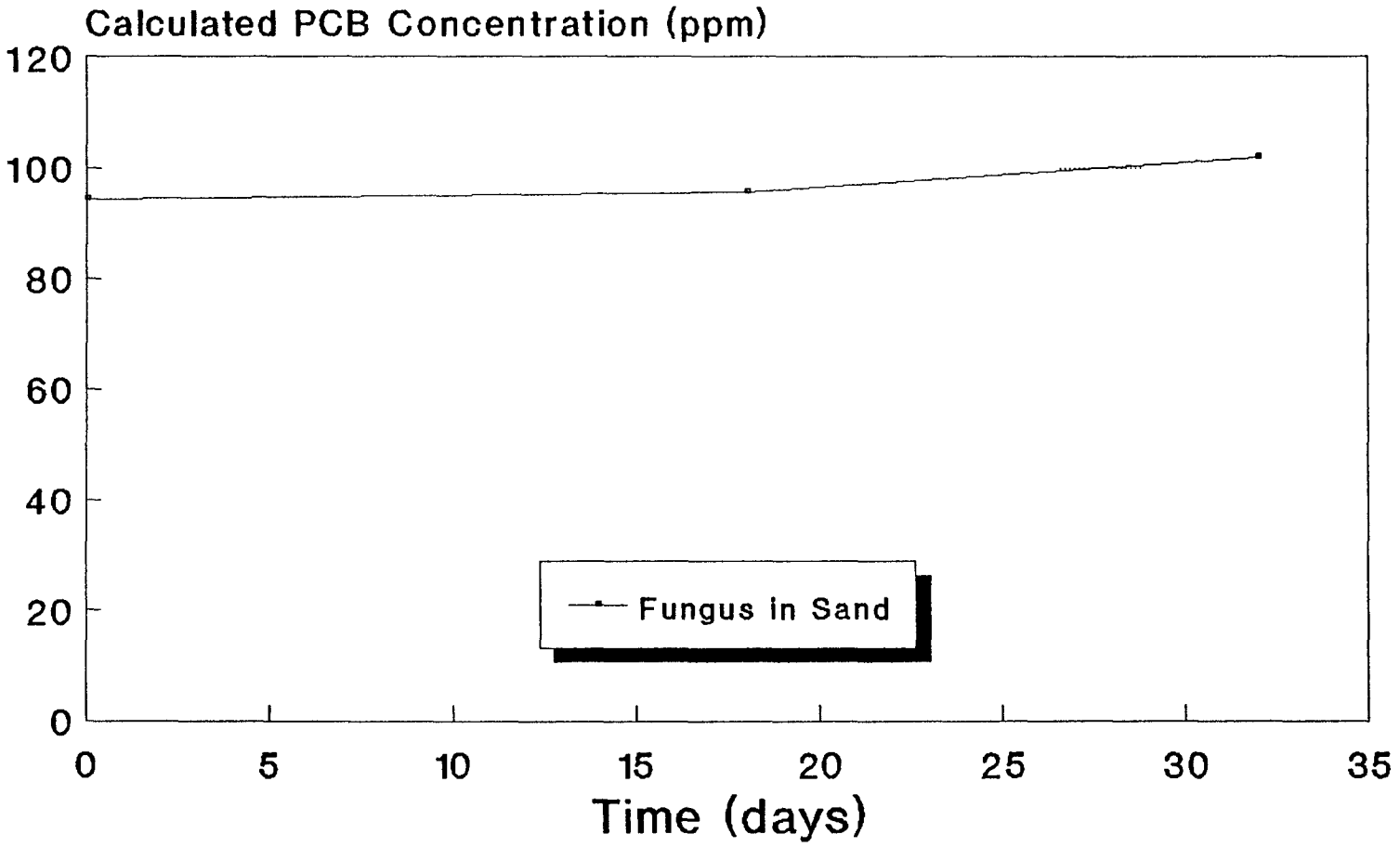
$$[\text{PCB in soil}] = [\text{PCB extracted}] / [1-\exp(-kt)]$$

where K is a given constant for a given system. This equation was then used to determine the amount of PCB in the sample from the amount of PCB recovered in the extract after a certain extraction time.

When such an analysis was performed, the amount of PCB theoretically recoverable from those bottles in which the PCB was exposed to the fungus was very similar to the amount originally added to the bottles. This is clearly shown in Figure 11 for Aroclor 1254 in sand. The equation was confirmed by the extraction tests performed with 1 ml of fungal inoculum (See Section 4.1.1.). In the extraction test, it was found that in these conditions, $K = 0.85\ days^{-1}$.

Similar negative results, as far as PCB degradation is concerned, were obtained for all the cultures, independently of the type of soil or sand used, the type of fungal addition (inoculum or biomass), or the concentration of nutrient in the system.

# Figure 11 Fungal Biodegradation Effectiveness Against Aroclor 1254 in Sand




Experimental Set # 1

In addition, similar negative results were also obtained for the other two types of Aroclors examined, i.e. Aroclor 1242 and Aroclor 1260. The corresponding chromatograms are shown in Figures 12 and 13, respectively.

In the experimental Set # 2, we wanted to see if instead of growing the fungus in the bottles (addition of 1 ml), we could add directly 15 ml of fungus taken in the best conditions. That way, the fungus could grow and biodegrade as it was the case in the former experiment. Some enzymes already present in the fungal inoculum might also be active in the added solution. Unfortunately, the results were exactly the same as what they were in the first set of experiments. No degradation, that is to say, no variation in the relative peak heights of the chromatograms was detected.

## 4.3. Experimental Set # 3

Since no degradation of PCBs was observed in the former experiments, we decided to use a control chemical to determine whether the fungus was active at all in the soil. TCP was chosen as a model target compound because it is known to be biodegradable by *P. chrysosporium*. Some control experiments without any fungus were also done. The results are presented in Tables 17 for Aroclor 1254 in soil and in Table 18 for TCP in soil. Some results with TCP in sand are presented in Table 19. Figure 14 shows the results for PCBs in soil, Figure 15 shows TCP in soil and Figure 16 shows the results for TCP in sand.

# Chromatograms of Aroclor 1242 Extracted from Soil

From Experimental Set # 1

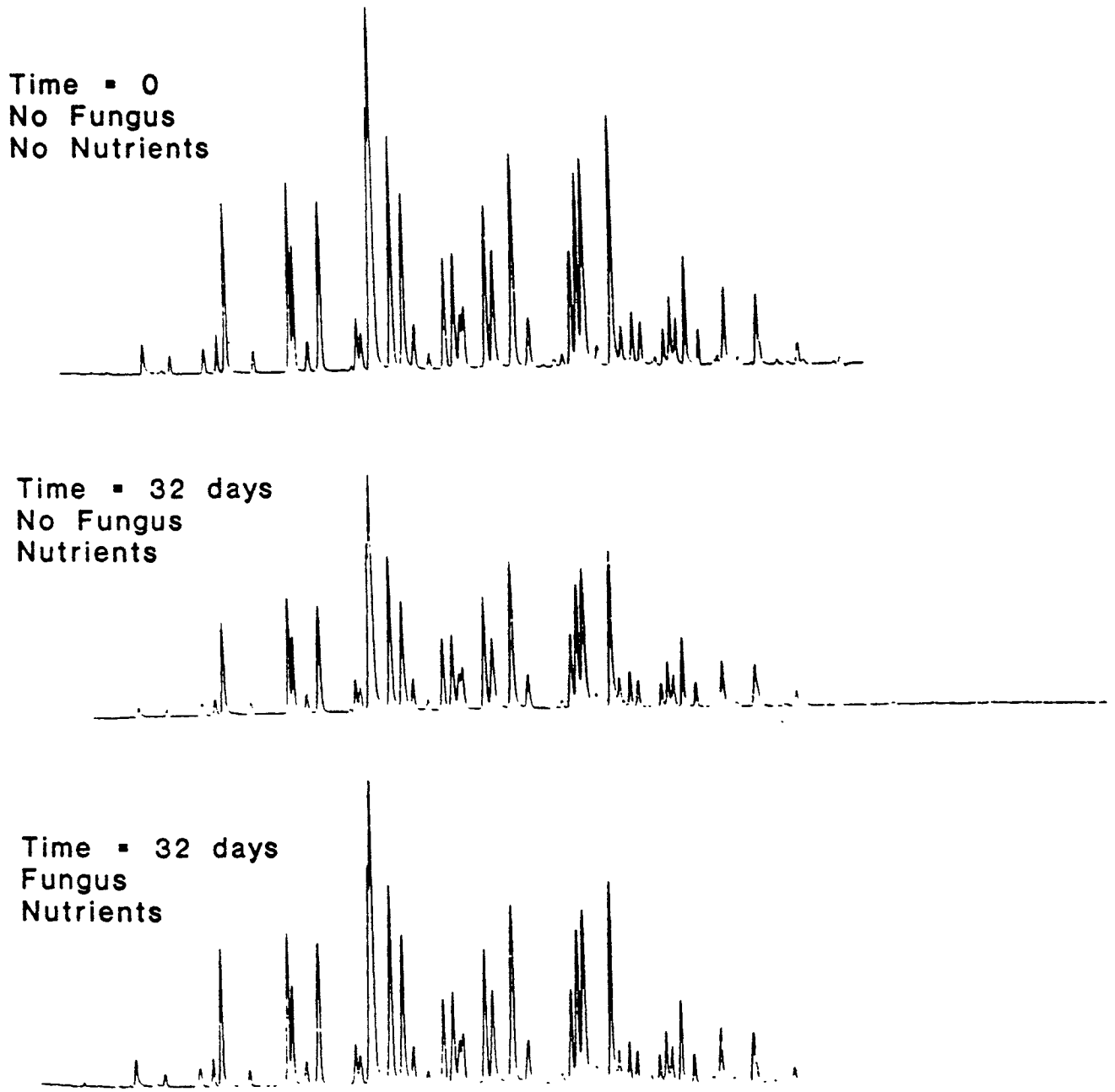



Figure 12

# Chromatograms of Aroclor 1260 Extracted from Soil

From Experimental Set # 1

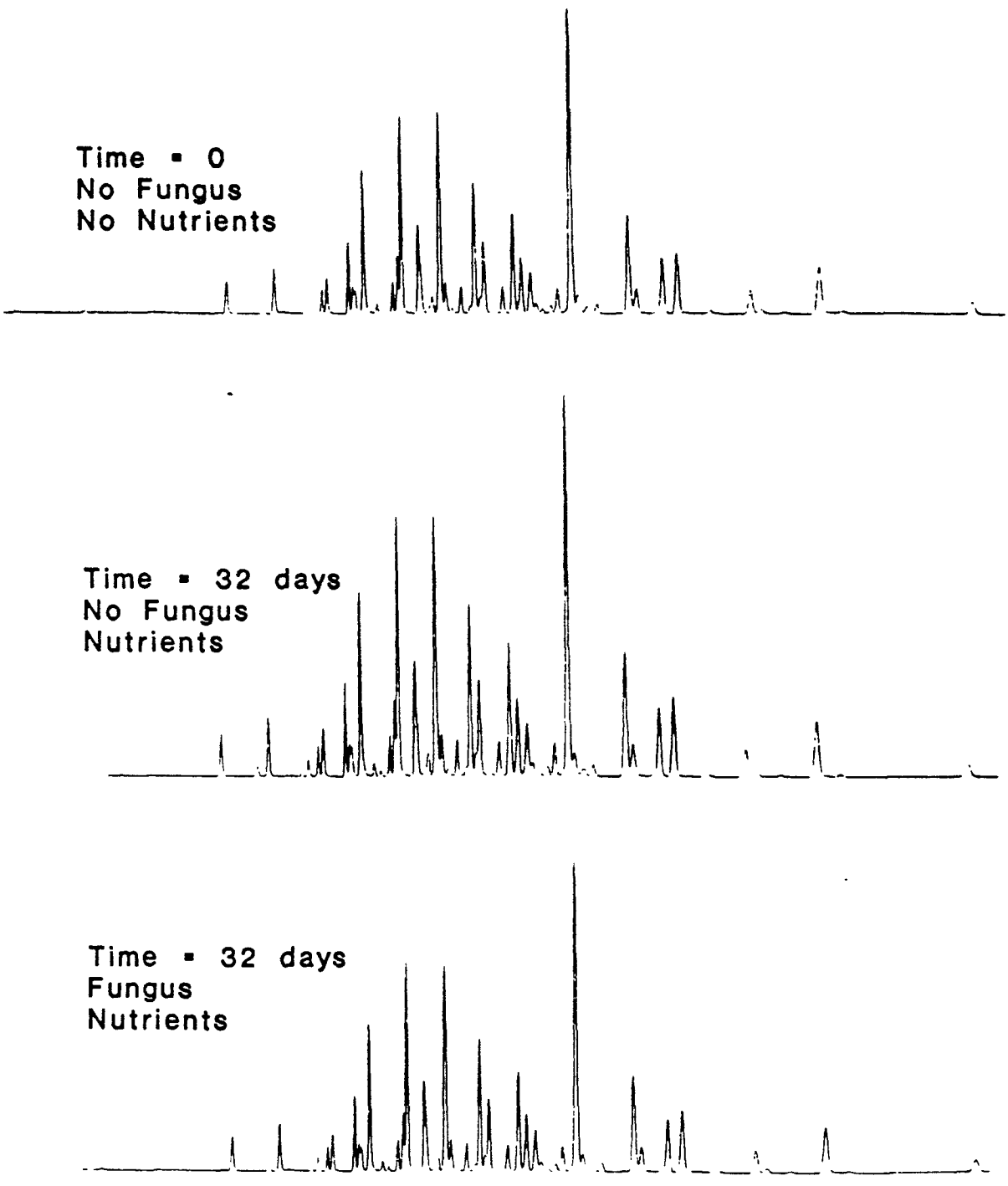



Figure 13

## Table 17 - Experimental Set # 3 with Aroclor 1254

(20 g of soil, with 20 ml fungal inoculum and additional air)

| Time (days)) | Control | | Experiment | |
|---|---|---|---|---|
| | Concentration of Aroclor 1254 (ppm) | % recovery | Concentration of Aroclor 1254 (ppm) | % recovery |
| 0 | 63.6 | 64 | 59.8 | 60 |
| 7 | 63.6 | 64 | 56.3 | 56 |
| 14 | 62.3 | 62 | 55.3 | 55 |
| 21 | 61.3 | 61 | 60.9 | 61 |
| 28 | 59.4 | 59 | 59.0 | 59 |

## Table 18 - Experimental Set # 3 with TCP in soil

(20g of soil, with 20 ml fungal inoculum and addditional air)

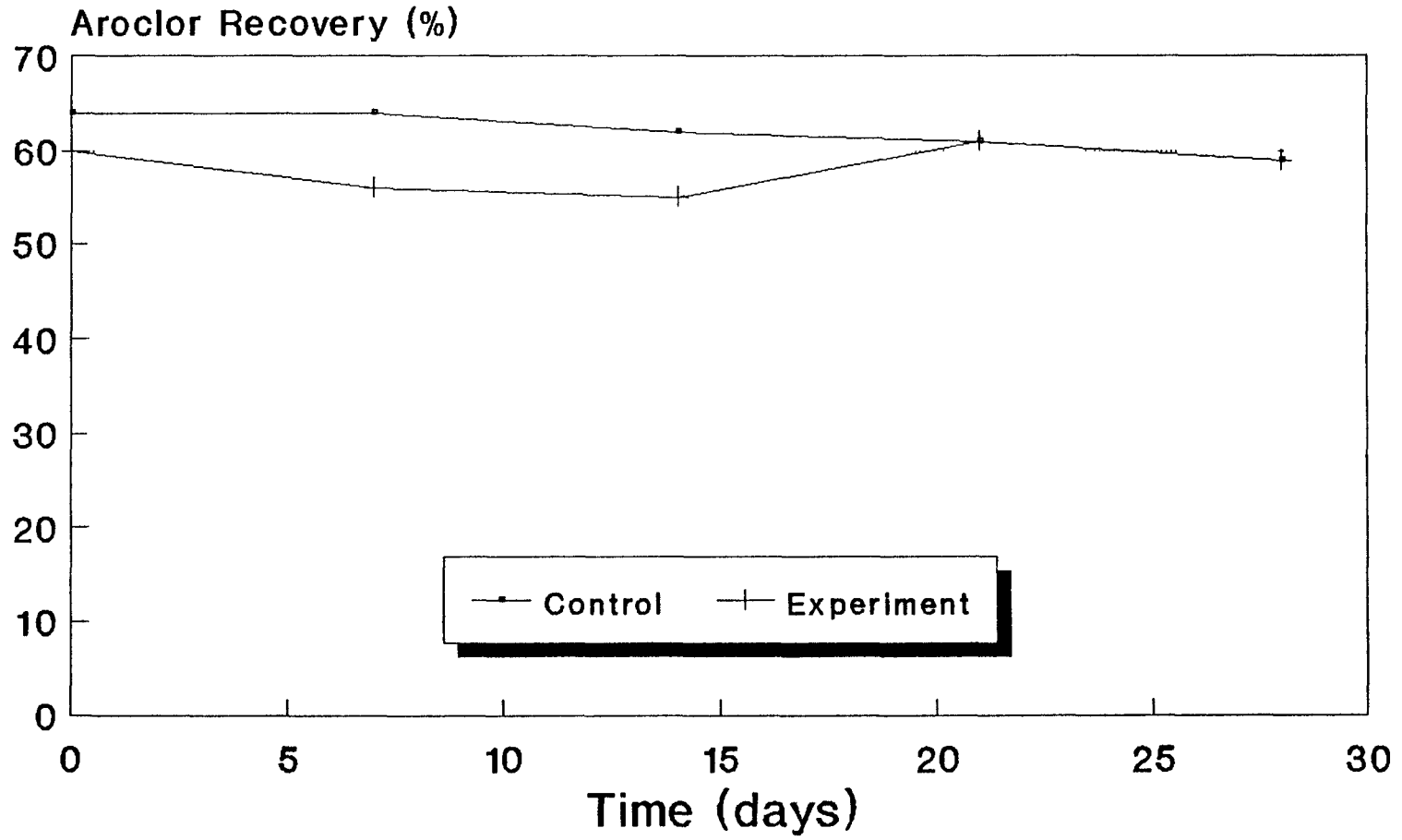
| Time (days) | Control | | Experiment | |
|---|---|---|---|---|
| | Concentration of TCP (ppm) | % recovery | Concentration of TCP (ppm) | % recovery |
| 0 | 81 | 81 | 82.1 | 82 |
| 3 | 78.5 | 78 | 71.5 | 72 |
| 7 | 83.1 | 83 | 73 | 73 |
| 10 | 78.8 | 79 | 69.6 | 70 |
| 14 | 80.7 | 81 | 52.7 | 53 |
| 17 | 76.9 | 77 | 48.4 | 48 |

## Table 19 - Experimental Set # 3 with TCP in Sand

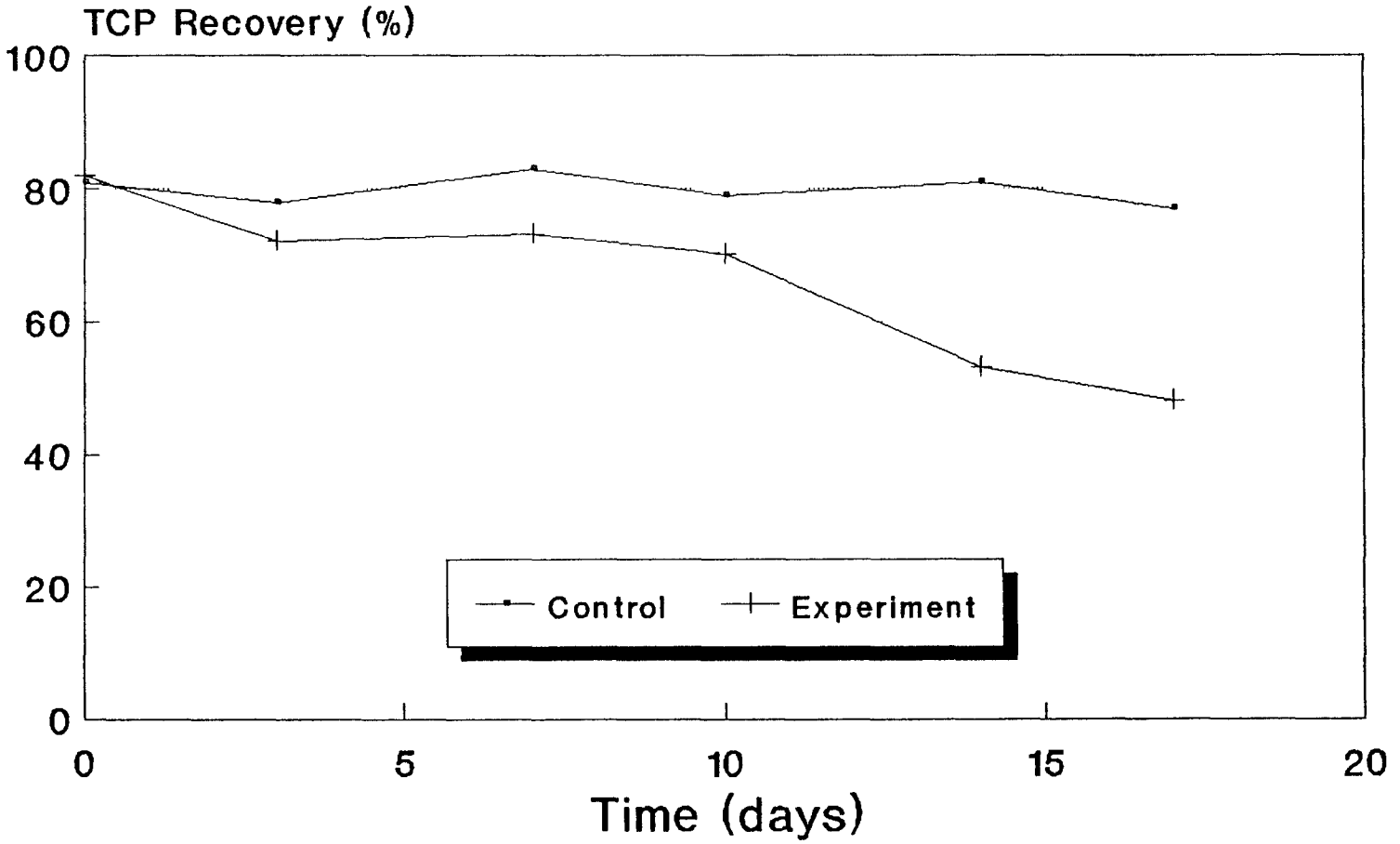
(20g of sand, 20ml of fungal inoculum.and additional air)

| Time (days) | Control | Experiment |
|---|---|---|
| | TCP Concentration (ppm) | TCP Concentration (ppm) |
| 0 | 53.8 | 53.3 |
| 3 | 38.9 | 38.1 |
| 6 | 53.9 | 17.4 |
| 9 | 39.7 | 7.4 |
| 12 | 53.0 | 3.3 |

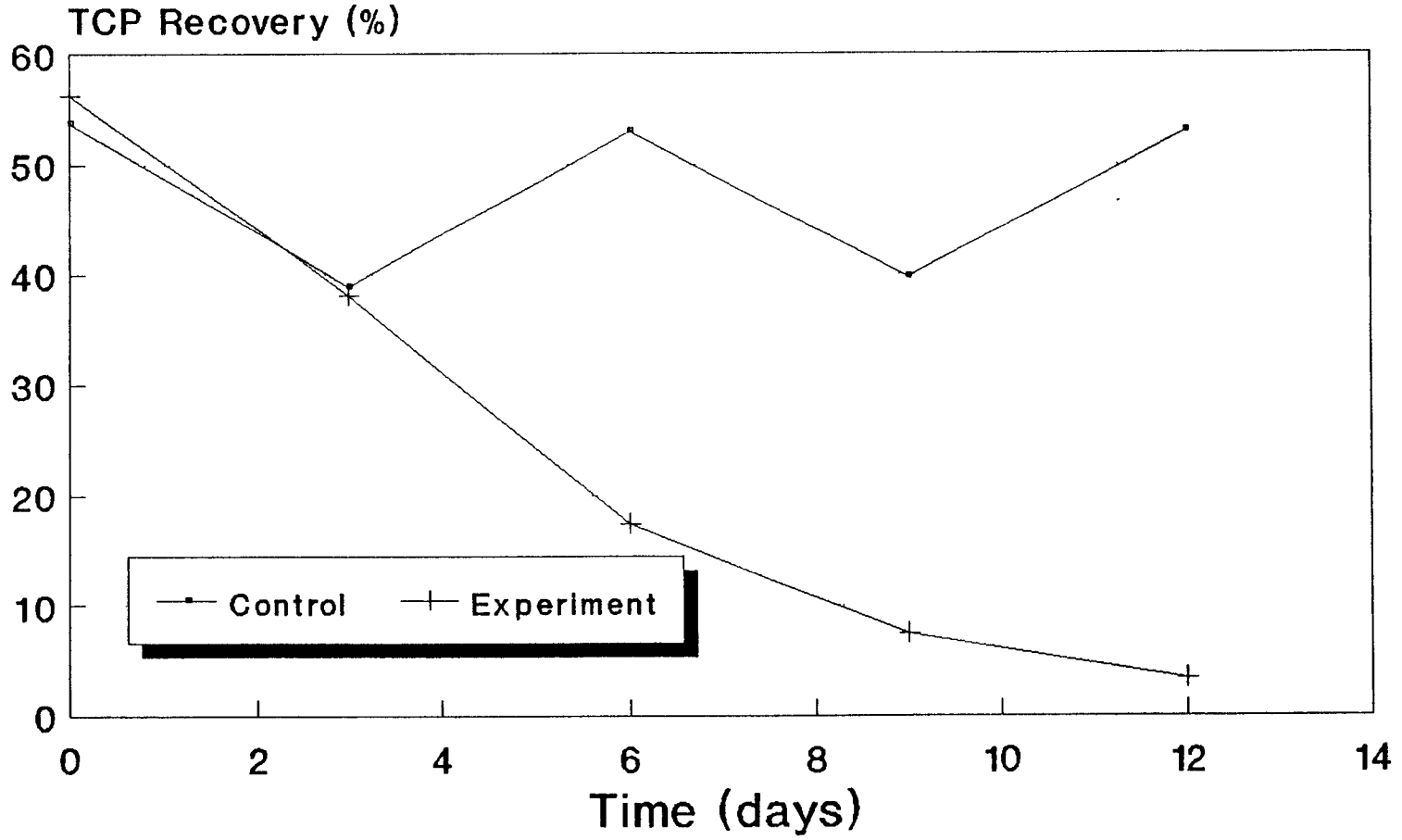
# Figure 14
# Experimental Set # 3 with Aroclor 1254 in Soil with 20 ml of Fungal Inoculun



# Figure 15
# Experimental Set # 3 with TCP in Soil with 20 ml of Fungal Inoculum



# Figure 16
# Experimental Set # 3 with TCP in Sand with 20 ml of Fungal Inoculum



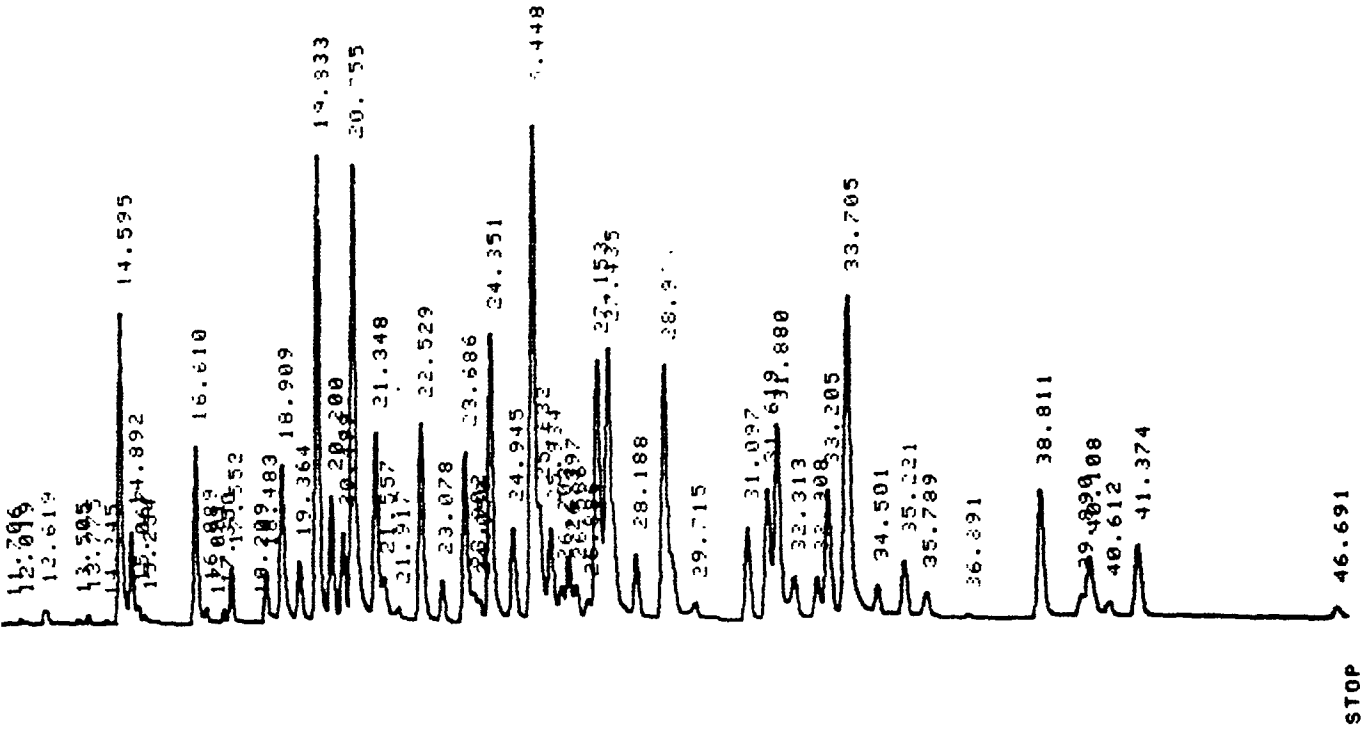
### 4.3.1. Results of PCB Degradation Experiments

The results shown in Figure 14, 15 and 16 indicate that no degradation on PCBs occurred, neither in soil nor in sand. There was no differences in the relative peak heights of the chromatograms. The chromatograms looked exactly alike. For example, Figure 17 shows a control chromatogram and an experimental one at time 0. Figure 18 shows a control and an experimental chromatogram at time 28 days. No differences can be detected between the chromatograms for the control experiment and the chromatograms for the experiment with the fungus. Similarly, no differences can be detected between the chromatograms at time 0 and at time 28 days. The same observations were made for time 7 days, 14 days and 21 days. The chromatograms (controls and experiments) for time 7, 14 and 21 days are presented in the appendix.

Furthermore, there is no differences between the PCBs concentration in both control and the actual experiment for time 28 days (59.4 ppm for the control and 59.0 ppm for the experimental sample).

## Comparison of the control chromatogram and the one from the experiment at time 0

### Control - Time 0




### Experiment - Time 0

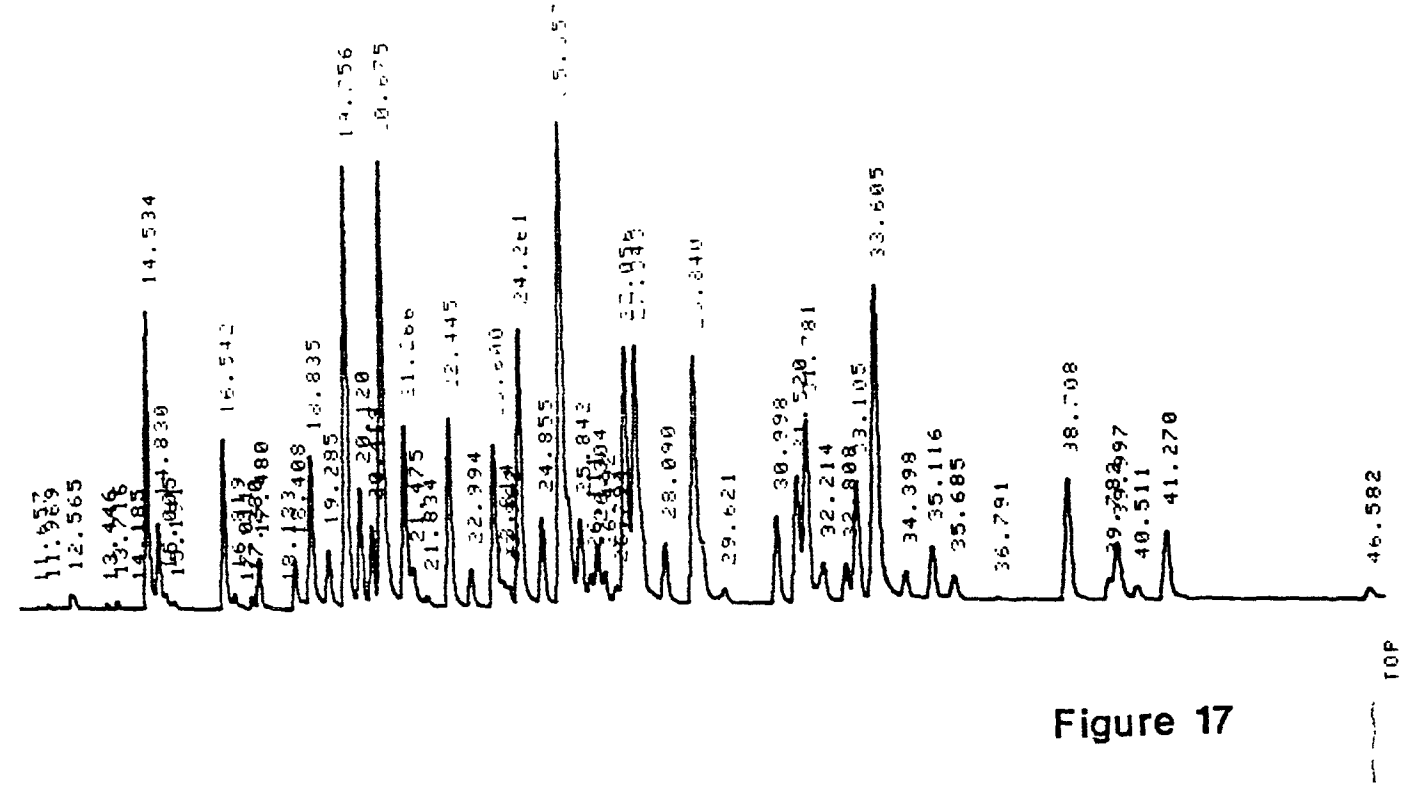
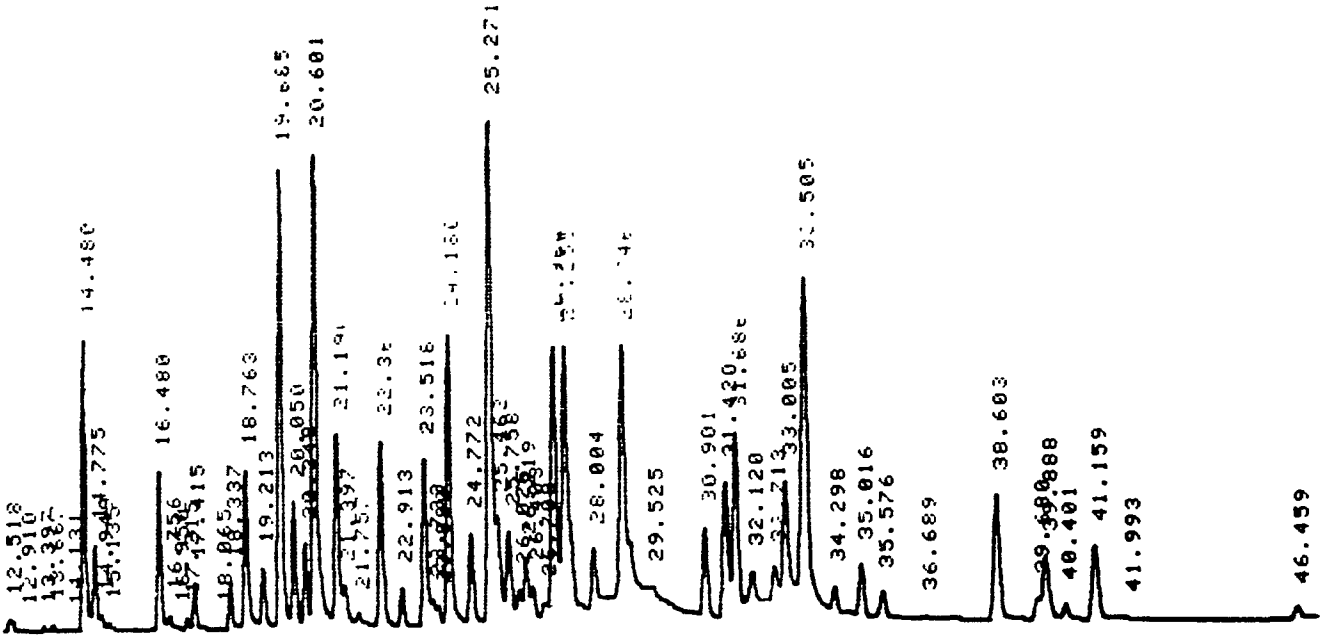



Figure 17

**Comparison of the control chromatogram and the one from the experiment at time 28 days**

**Control - Time 28 days**




**Experiment - Time 28 days**

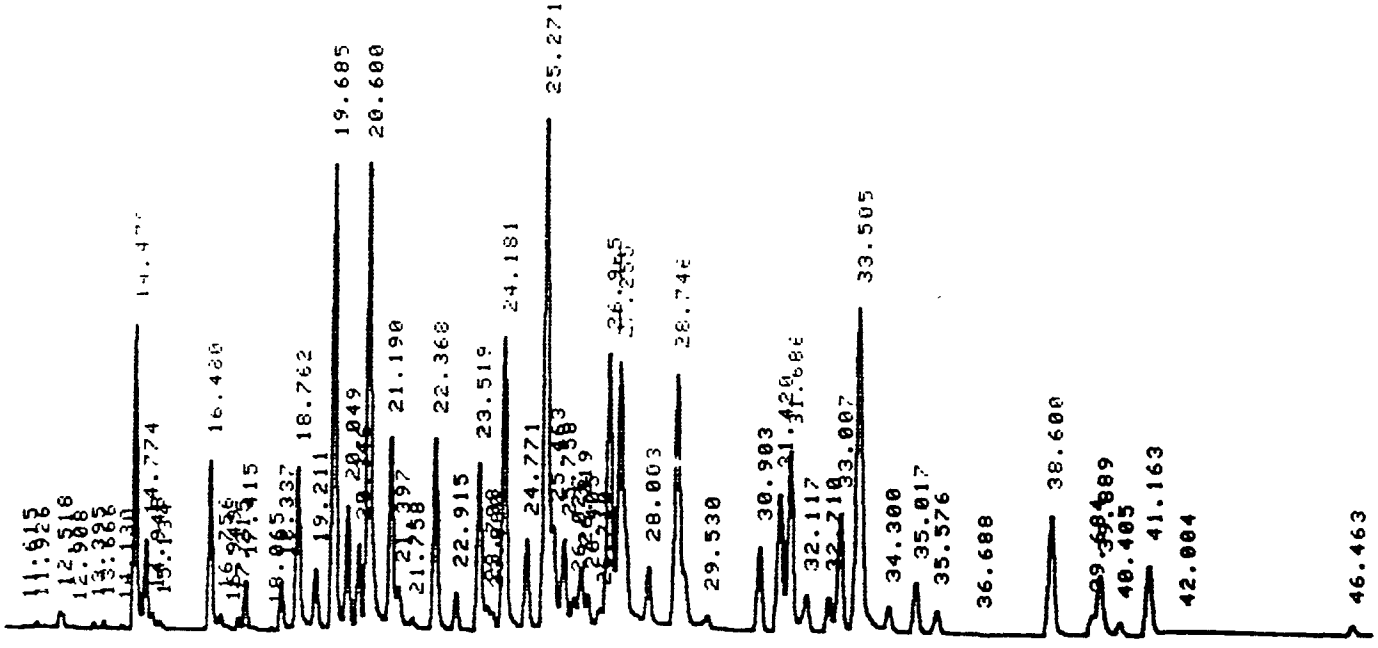



Figure 18

### 4.3.2. Results of TCP Degradation Experiments

It is difficult to say if there was a degradation of TCP in soil. From time 0 to time 10 days, no degradation was observed (no concentration differences could be observed between the controls and the experiments). From time 10 days to the end of the experiment (17 days), the experimental sample had a lower concentration than that observed for the controls. The controls remained around 80 ppm while the experiment sample concentration decreased from 69.6 ppm at 10 days, to 52.7 ppm at 14 days and 48.4 ppm at time 17 days. The decrease is too low to say without doubt if a degradation occurred. Even if some degradation took place it does not appear to be significant, and further experiments are necessary to verify this point.

However, the results in sand show a clear TCP degradation. In 12 days, the concentration decreased from 55 ppm at time 0 to 3.3 ppm at time 12 days. This is equivalent to a 94% degradation. In the meanwhile, the controls have remained at their initial concentrations. The degradation of TCP came from the same original batch of fungal biomass used in the experiments with PCBs.

### 4.3.3. Recovery Problems with Experimental Set # 3

We have seen that no PCB degradation was observed. However, the PCB recovery was not as good as what we expected from the extraction experiments. We obtained 60 % recovery from the PCB experiments when 100 % was expected. The same phenomena occurred with TCP. 80 % recovery was obtained instead of 95.5 % expected. This was observed in both control and experimental samples.

During the experiments, the contaminants remained longer in contact with the soil. During that additional period of time, the contaminant might interact more strongly with the soil and therefore make the extraction more difficult.

Even if the extraction efficiency were lower than what we expected, the results seem to be quite homogeneous.

# Chapter 5 CONCLUSIONS

This work was conducted to determine the degradation of PCBs by *P. chrysosporium* in two different type of solid matrices: soil and sand. The soil that has been used is a "clean" EPA soil, i.e. it is not contaminated by any chemical pollutants. However, every soil has its own characteristics and composition. Therefore, the results obtained on soil cannot always be generalize. On the other hand, results with sand can be generalized.

During the experiments, The PCB recovery was not 100 %. However, with the chromatograms, comparing the relative peak heights, it was possible to conclude on the inability of the white rot fungus *Phanerochaete chrysosporium* to biodegrade PCBs. When a fungus was added to sand, it was capable of biodegrading TCP, but it was not capable of biodegrading PCBs. However, there is still a possibility that the fungus could need more time to biodegrade PCBs than it does to biodegrade TCP. An experiment conducted for a longer period of time would be interesting.

The results with soil were not so conclusive. No degradation was observed with PCBs. However, it is difficult to say if the decrease observed in the quantity of

TCP was due to a degradation. To determine if it is possible to get degradation of TCP in soil, further experiments would have to be done.

It is recommended that in future work, the following parameters should be monitored in the soil: chloride, nitrogen, glucose, protein and pH. These data might help us find soil conditions that could biodegrade the PCBs.

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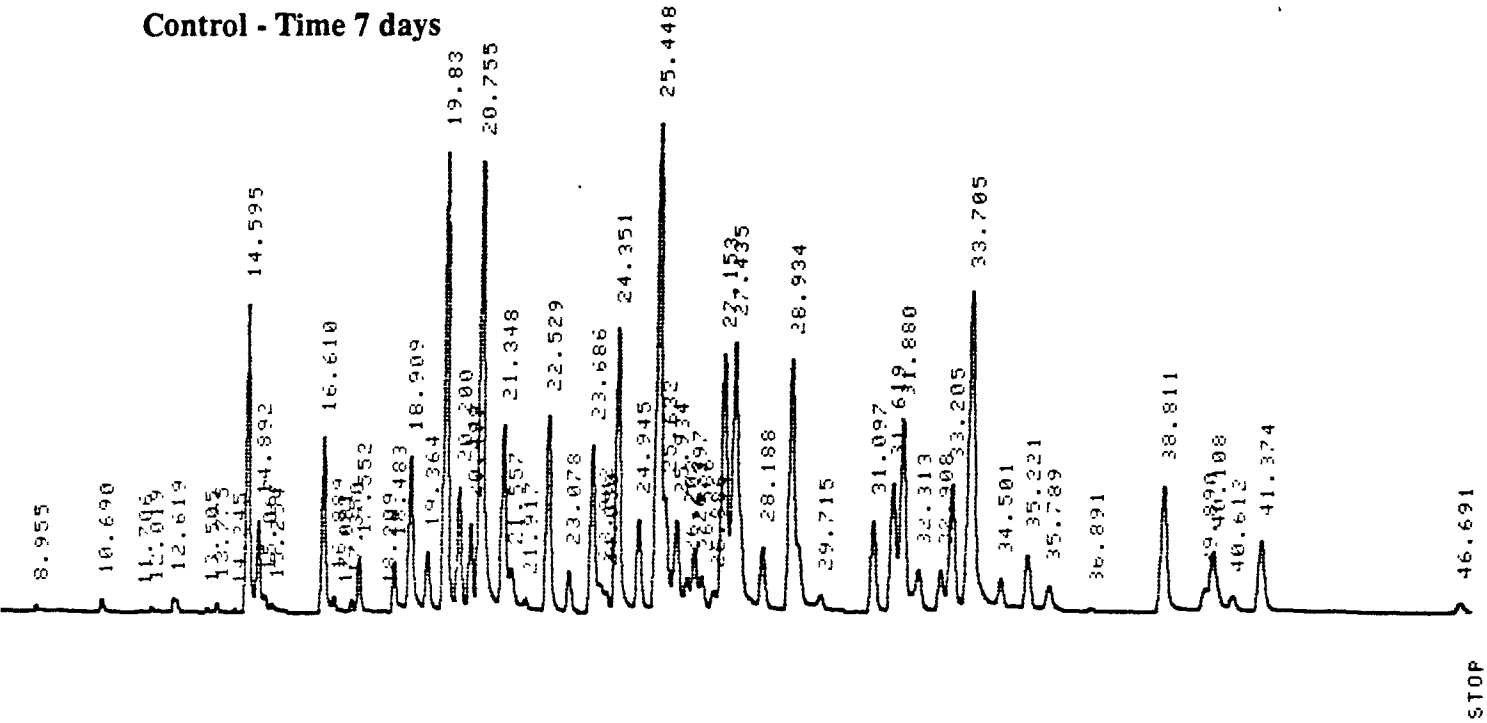
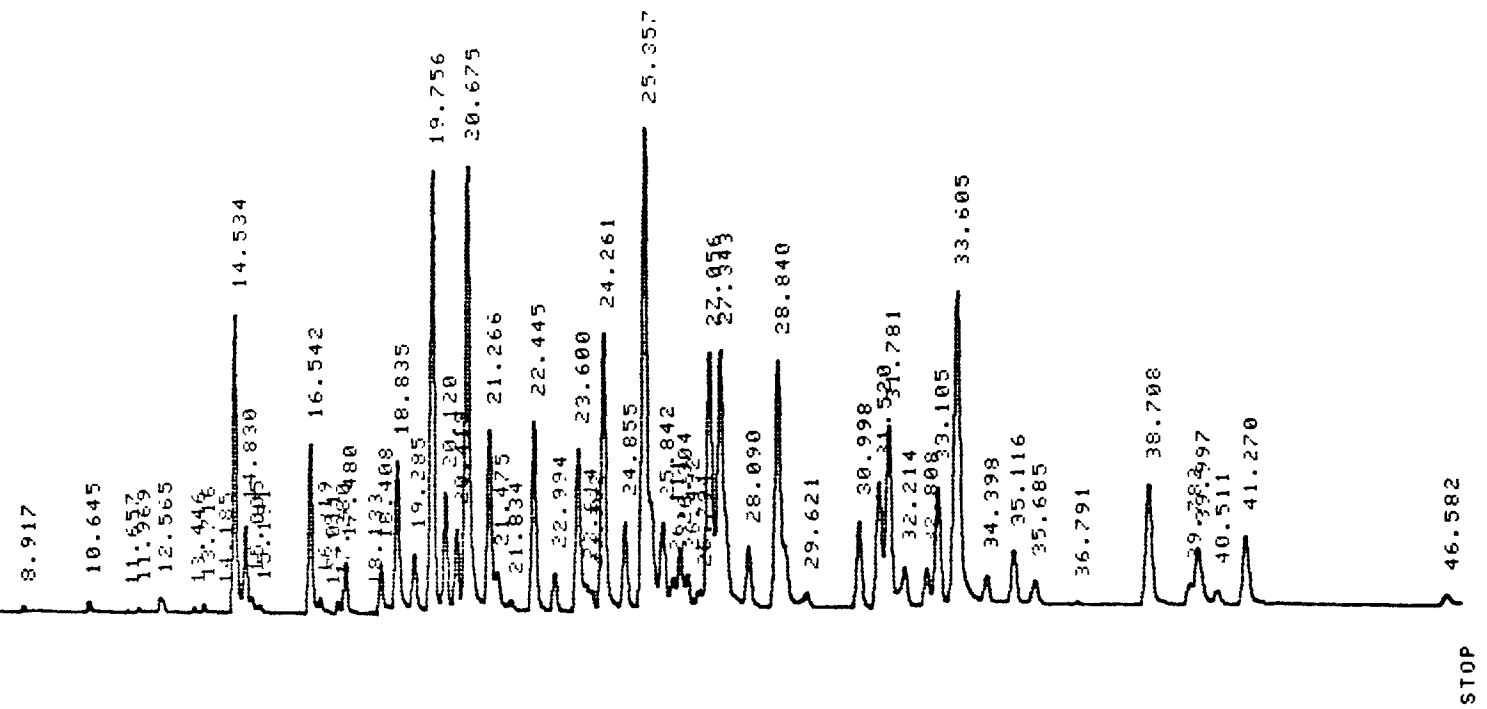
# Appendix

**Comparison of the Control Chromatogram and the One from the Experiment at Time 7 Days**

**Control - Time 7 days**

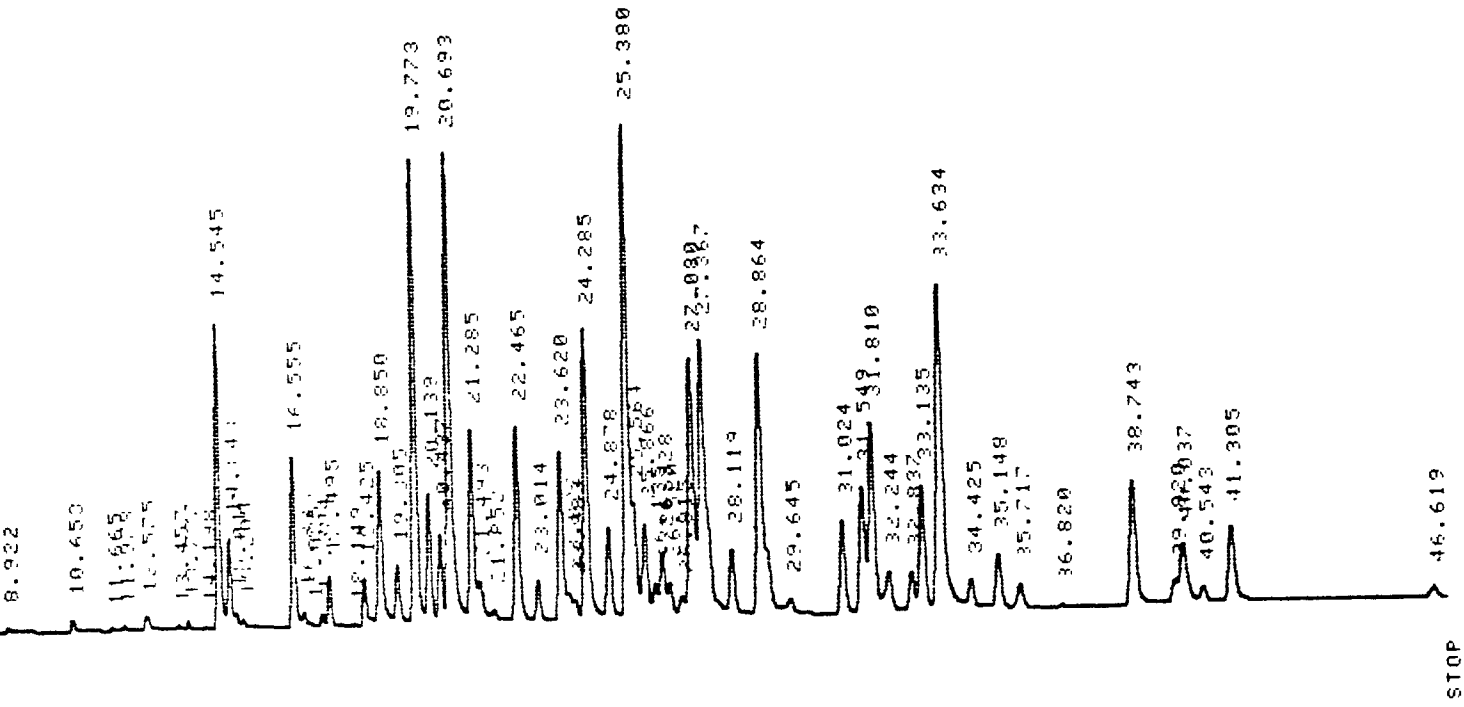



**Experiment - Time 7 days**

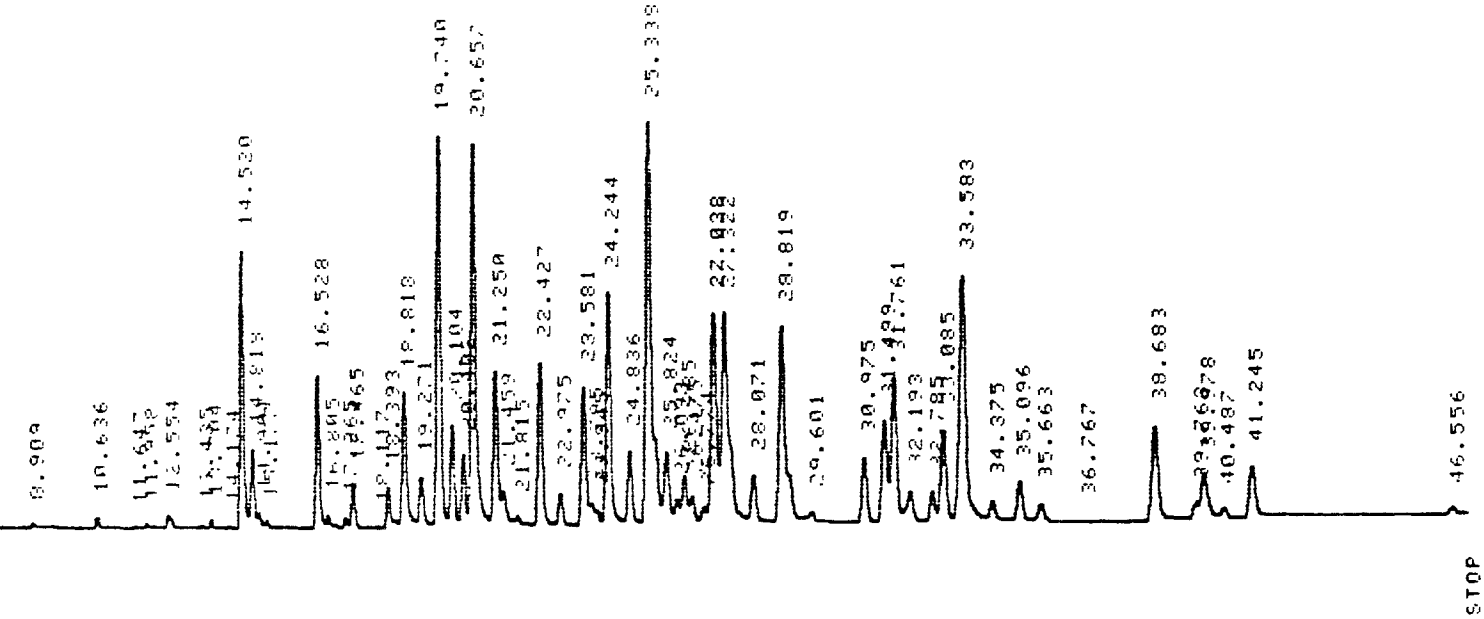


**Comparison of the Control Chromatogram and the One from the Experiment at Time 14 Days**

**Control - Time 14 days**

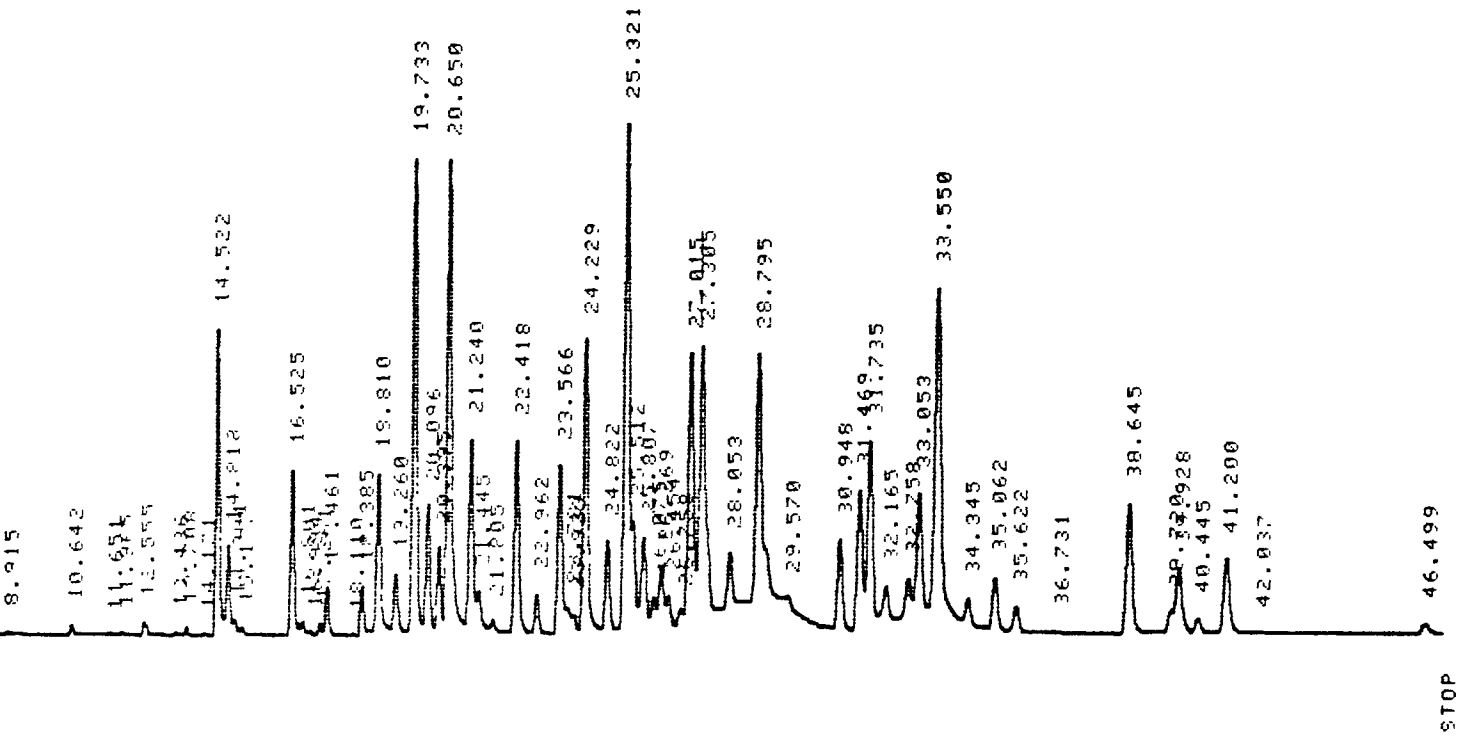



**Experiment - Time 14 days**



## Comparison of the Control Chromatogram and the One from the Experiment at Time 21 Days

### Control - Time 21 days




### Experiment - Time 21 days