AN ABSTRACT OF THE THESIS OF

Jon D. Polonsky for the degree of Master of Science in Civil Engineering presented on May 2, 1988.

Title: The Development of a Gas-Permeable-Membrane-Supported (GPMS) Biofilm Reactor for the Combined Anaerobic/Aerobic Treatment of Polychlorinated Biphenyls

Abstract approved: Redacted for Privacy
Sandra L. Woods

A continuous flow, gas-permeable-membrane-supported (GPMS) biofilm reactor was developed to facilitate the treatment of polychlorinated biphenyls (PCBs). The GPMS biofilm reactor consisted of a cylindrical glass vessel made up of three compartments: the gas, liquid and headspace compartments. A gas-permeable membrane separated the bottom gas compartment from the liquid compartment. The biofilm, comprised of a mixed consortia of aerobic and anaerobic microorganisms, was grown on the liquid side of the membrane, and was supplied with oxygen from the gas compartment. Chemical oxygen demand (COD) analyses of reactor liquid indicated that it was possible to maintain a relatively constant biomass over time.

PCBs were removed from the reactor influent by the GPMS biofilm reactor at efficiencies of 82 to 99 percent, depending on the PCB influent concentration and the degree of chlorination. The major removal mechanism involved adsorption of PCBs to biomass in the reactor. Gas chromatography-electron capture detector and gas chromatography-mass spectrometry analyses of biofilm samples suggested

that 2,3,4,5,6-pentachlorobiphenyl was reductively dechlorinated to produce 2,3,5,6-tetrachlorobiphenyl. Thus, the process resulted in the degradation of one of the parent compounds by removing a chlorine from the para position.

The Development of a Gas-Permeable-Membrane-Supported (GPMS) Biofilm Reactor for the Combined Anaerobic/Aerobic Treatment of Polychlorinated Biphenyls

by

Jon D. Polonsky

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
degree of

Master of Science

Completed May 2, 1988

Commencement June 1988

APPROVED:

Redacted for Privacy

Professor of Civil Engineering in charge of major

Redacted for Privacy

Head of department of Civil Engineering

Redacted for Privacy

Dean of Graduate School

Date thesis is presented May 2, 1988

ACKNOWLEDGEMENTS

I appreciate the opportunity to have worked on this project, as well as the advice given to me by Professors Sandra Woods and Kenneth Williamson. Funding provided by the Environmental Protection Agency for GPMS biofilm reactor research proved crucial in obtaining necessary equipment and supplies for this project. Information provided by General Electric Company was another vital asset in developing my background knowledge in the field of PCB biodegradation. Detailed analyses of reactor samples using GC/MS was possible only with the valuable assistance of Fred Roberts, at the Mark O. Hatfield Marine Sciences Center, Environmental Protection Agency Laboratory in Newport, Oregon.

I also would like to thank my friends, McDuff, Clackamos and Vicky for their patience and love during the completion of this research project. Without their presence and advice, this study may not have been completed.

Table of Contents

CHAPTER ONE 1

INTRODUCTION & LITERATURE REVIEW 1

MATERIALS & METHODS 7

The Reactor 7

Analytical Methods 13

RESULTS 17

COD Results 17

Anaerobic Results 19

PCB Concentrations in Reactor Liquid 19

PCB Removal 25

Metabolite Analysis 25

DISCUSSION 32

Steady State 32

Anaerobic Discussion 32

PCB Concentrations in the Reactor Liquid 32

PCB Removal 33

SUMMARY & CONCLUSIONS 36

Conclusions 37

CHAPTER TWO 38

SIGNIFICANCE & RECOMMENDATIONS 38

LITERATURE CITED 41

APPENDICES 43

List of Figures

1. PCBs used in reactor feed 6

2. GPMS biofilm reactor design schematic 8

3. Chemical oxygen demand in reactor liquid 18

4. Concentration of 2,3'4,4'-CB in reactor effluent 20

5. Concentration of 2,3,4,5,6-CB in reactor effluent 21

6. Concentration of 2,2'4,4'6,6'-CB in reactor effluent 22

7. Concentration of 2,2'3,4,4'5'6-CB in reactor effluent 23

8. Concentration of 2,2'3,3'4,4'5,5'-CB in reactor effluent . . . 24

9. GC/ECD chromatograms of reactor feed and reactor liquid sample taken at day 220 28

10. GC/ECD chromatograms of biofilm samples taken on day 165 and day 220 29

11. Mass spectrums of Peak B and 2,3,5,6-CB 31

List of Tables

1. Nutrient Feed Solution 10
2. PCB Stock and Feed Concentrations 12
3. Reactor Feed Periods 12
4. Average PCB Percent Removal 26

PREFACE

Polychlorobiphenyl (PCB) contamination of the environment has occurred at sites all over the world (Mackay et al., 1983; Higuchi, 1976). Because chlorinated biphenyls have been shown to have adverse effects on living forms, this hazardous waste is a major concern to those who wish to have a reasonably safe environment. Although the use of PCBs has greatly diminished, their stability in the environment has resulted in the need for treatment processes to eliminate PCBs from the air, soil and water.

Much research has been performed to identify bacteria which are capable of biodegrading PCBs to less toxic forms. Evidence has been found that certain chlorinated biphenyls with less than six chlorines are degraded by some aerobic microorganisms (Bailey et al., 1983, Bedard et al., 1986 and 1987, Furukawa et al., 1983). Aerobic microorganisms utilize enzyme mediated hydroxylation and ring cleavage reactions to metabolize PCBs.

Less information is available regarding anaerobic degradation of PCBs. Anaerobic degradation involves reductive dechlorination reactions. Using an anaerobic upflow biofilter, Low and Woods (1988) observed dechlorination of 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-CB) to 2,3,5,6-CB. In anaerobic batch studies, Tiedje et al. (1987) obtained similar dechlorination results involving 2,3,4,5,6-CB as well as the reductive dechlorination of 2,2'3,4,4'5'6-CB to 2,2'4,4'5,6'-CB.

The study presented in the following chapter was performed with the use of a group of five PCBs, ranging from tetra- to octachlorobiphenyl, and a GPMS biofilm reactor.

The objectives of this project were:

1. to cultivate a PCB-acclimated consortia of anaerobic and aerobic microorganisms in a GPMS biofilm reactor,
2. to develop a steady state continuous flow GPMS biofilm reactor with respect to biomass, COD removal, and pH,
3. to demonstrate the removal of PCBs from PCB-contaminated water using a GPMS biofilm reactor, and
4. to determine anaerobic/aerobic pathways of PCB biodegradation.

In the following chapter, this study is presented in the form of a technical manuscript. It includes a more detailed introduction and literature review, as well as sections on materials and methods used, results, discussion, and a summary and conclusion. Chapter 2 includes a discussion of the significance of this experiment, recommendations for future study, and a list of references. Data collected concerning COD, PCB concentrations, and pH can be found in the appendices.

CHAPTER ONE

The Development of a Gas-Permeable-Membrane-Supported (GPMS) Biofilm Reactor for the Combined Anaerobic/Aerobic Treatment of Polychlorinated Biphenyls

INTRODUCTION & LITERATURE REVIEW

The presence of polychlorinated biphenyls (PCBs) in the environment represents a serious toxicological and ecological problem. Due to their relatively slow degradation in water, air and soil systems, the accumulation of these toxic compounds in the world's ecosystems poses a potential threat to the health of all living things. PCBs were used in the United States of America for fifty years until their use was outlawed in 1977. While their use has been dramatically reduced worldwide, their contamination lingers due to their stability in natural environments.

The basic structure of a biphenyl molecule consists of two benzene rings joined by a single carbon-carbon bond. There are 209 congeners of chlorinated biphenyls that can result from the presence of one to ten chlorines in various positions on a biphenyl.

PCBs are relatively insoluble in water and have strong adsorptive properties. The aqueous solubility ranges between 0.00049 mg/l for decachlorobiphenyl (Yalkowsky et al., 1983) to 5.9 mg/l for a monochlorobiphenyl (Hutzinger et al., 1974). The log octanol/water partition coefficient (log K_{OW}) varies between 4.6 for a monochlorobiphenyl to 9.6 for decachlorobiphenyl (Bruggenman et al., 1982). The low aqueous solubilities and high partition coefficients

of PCBs result in a strong association of PCBs with organic solids in the environment and in biological reactors.

Little research has been done concerning anaerobic biodegradation of PCBs. Anaerobic degradation involves a reductive dechlorination reaction in which PCBs may act as electron acceptors and are reduced (Brown et al., 1987). In anaerobic upflow biofilter experiments, Low and Woods (1988) found evidence of reductive dechlorination which resulted in the transformation of 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-CB) to 2,3,5,6-CB. Tiedje et al. (1987) performed anaerobic bottle tests which produced similar dechlorination of 2,3,4,5,6-CB, as well as dechlorination of 2,2'3,4,4'5'6-CB to 2,2'4,4'5,6'-CB. Comparison of chromatograms from Aroclors and Aroclor-contaminated anaerobic sediments of the Hudson River and Silver Lake (Massachusetts) showed an increase in the concentration of mono- and dichlorobiphenyls, and a decrease in the concentration of higher chlorinated biphenyls (Brown et al., 1987). This suggested that higher chlorinated PCBs may have been dechlorinated to produce lower chlorinated PCBs. Several conceivable patterns of elimination were observed by Brown et al. in which sediments at one site resulted in the selective removal of chlorines meta and para to the single carbon-carbon bond, while contaminated sediments at another site resulted in removal of chlorines ortho, meta and para to the single carbon-carbon bond (Brown et al., 1987).

Aerobic degradation of PCBs involves hydroxylation and ring cleavage reactions. In these reactions, PCBs may be either cometabolized or used as electron donors, with oxygen serving as the electron acceptor. The hydroxylation reactions are mediated by

monooxygenase or dioxygenase enzymes which may result in one or two hydroxyl (-OH) groups being attached to the PCB (Bedard et al., 1987; Furukawa et al., 1983). Due to the addition of the hydrophilic hydroxyl groups, these polychlorinated biphenylols and biphenyldiols are more water soluble and may be more readily biodegraded than the unhydroxylated PCBs.

The number of adjacent unchlorinated sites can be a factor in determining the aerobic degradability of PCBs. This may be due to the requirement of the dioxygenase enzyme for two adjacent unchlorinated sites on the biphenyl to hydroxylate the compound (Bedard et al., 1987; Masse et al., 1984). Aerobic degradation by a dioxygenase pathway is less likely to occur to highly chlorinated PCBs because of the less common occurrence of adjacent unchlorinated sites. In addition, some species of bacteria have been observed to hydroxylate PCBs which are unchlorinated in the 2,3 positions, while other aerobes hydroxylated PCBs unchlorinated at the 3,4 positions (Bedard et al., 1987).

Because different aerobic and anaerobic pathways may result in the metabolism of PCBs, it may be advantageous to utilize a mixture of microorganisms capable of both aerobic and anaerobic pathways to more completely degrade PCBs. This could be done by sequentially treating PCB-contaminated wastewater anaerobically to facilitate PCB dechlorination, followed by aerobic treatment to promote hydroxylation and further breakdown of the PCBs to less toxic compounds. Another possibility would be a process which combines both anaerobic and aerobic bacteria in one reactor. A combined process was studied in this project using a gas-permeable-membrane-supported (GPMS) biofilm reactor.

A traditional biofilm involves the growth of microorganisms fixed to a solid surface. Quite often, this biofilm is in contact with a liquid phase which may contain oxygen. If the liquid does contain oxygen, bacteria at the liquid/biofilm interface will be aerobic. As the biofilm grows deeper, diffusion of oxygen may become limited such that microorganisms growing deep within the biofilm are anaerobic.

The GPMS biofilm is similar to a traditional biofilm in that it grows attached to a fixed surface and is in contact with a liquid phase. The main difference in the GPMS biofilm is that the fixed surface is a gas-permeable membrane. The biofilm grows on the liquid side of the gas-permeable membrane. By supplying oxygen to the gas side of the membrane, an aerobic layer of growth occurs at the biofilm/membrane interface. As the biofilm grows deeper, and if the liquid phase is closed to the atmosphere, an anaerobic zone of growth may develop at the biofilm/liquid interface.

The PCBs chosen for this study were selected because of their wide range of substitution patterns and the ability to detect each compound and it's potential first dechlorinated metabolic product by gas chromatographic analyses (Figure 1). Varying combinations of ortho, meta and para chlorinated PCBs are present in this group which allows a wide range of potential anaerobic degradation pathways. The tetra- and pentachlorobiphenyls have several adjacent unchlorinated sites which may be susceptible to aerobic degradation by the dioxygenase enzyme pathway. Similarly, dechlorinated products of the hexa-, hepta- and octachlorobiphenyls may also be aerobically degraded by the dioxygenase pathway.

Utilizing the group of PCBs in Figure 1 and a GPMS biofilm reactor, the objectives of this study were:

1. to cultivate a PCB-acclimated consortia of anaerobic and aerobic microorganisms in a GPMS biofilm reactor,
2. to develop a steady state continuous flow GPMS biofilm reactor with respect to biomass, COD removal, and pH,
3. to demonstrate removal of PCBs from PCB-contaminated water using a GPMS biofilm reactor, and
4. to determine anaerobic/aerobic pathways of PCB biodegradation.

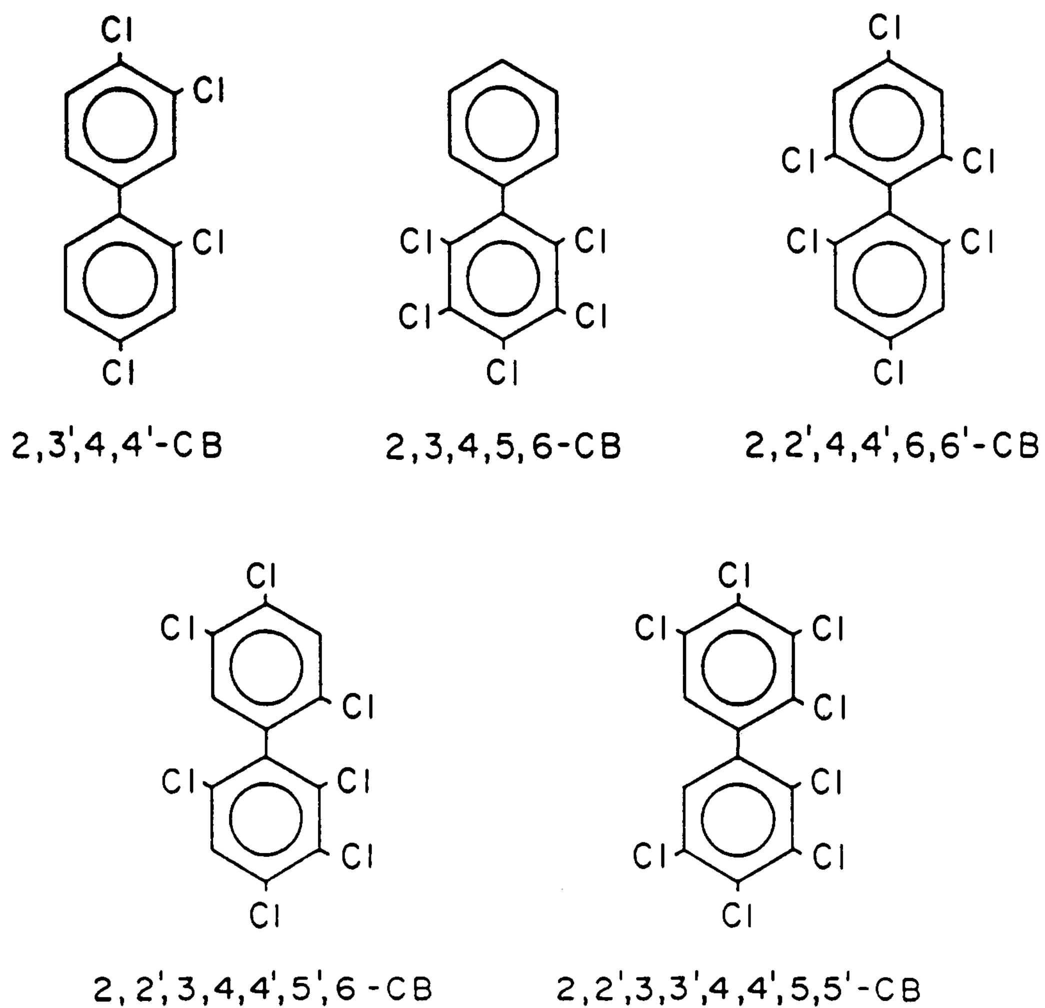


Figure 1. PCBs in reactor feed

MATERIALS & METHODS

The Reactor

The GPMS biofilm reactor was constructed of Kimax beaded glass process pipe (Figure 2). It was 57 cm in height and 10.2 cm in diameter. It consisted of three sections: the gas, liquid, and headspace sections. The sections were joined by stainless steel fittings with Teflon flanges. A Goretex membrane (W.L.Gore & Associates, Elkton, MD) was stretched between the joint of the liquid section and the gas reservoir. The liquid phase was supported by the membrane and the biofilm was grown on the liquid side of the membrane. The bacteria used to seed the initial biofilm were obtained from the Corvallis, Oregon municipal wastewater treatment plant. The reactor was incubated at 32°C throughout the experiment.

Oxygen was supplied to the gas reservoir at a constant pressure of 5 inches of water using a low-pressure regulator (Matheson Gas Products Inc., Newark, CA). Oxygen was used to provide aerobes in the biofilm with an electron acceptor, and to support the membrane and the five to six inches of reactor liquid. To insure a uniform supply of oxygen to the biofilm, the gas reservoir was mixed continuously using a magnetic stir bar and mixer.

The liquid phase was constantly mixed by a Teflon paddle attached to a Teflon coated borosilicate glass stirring rod (Ace Glass, Vineland, NJ) which was driven at 60 rpm by a small electric motor (Minarik Electric Co., Los Angeles, CA).

The nutrient feed solution (minus the PCBs/acetone solution) was held at 4°C in a four-liter flask. It was pumped into the reactor

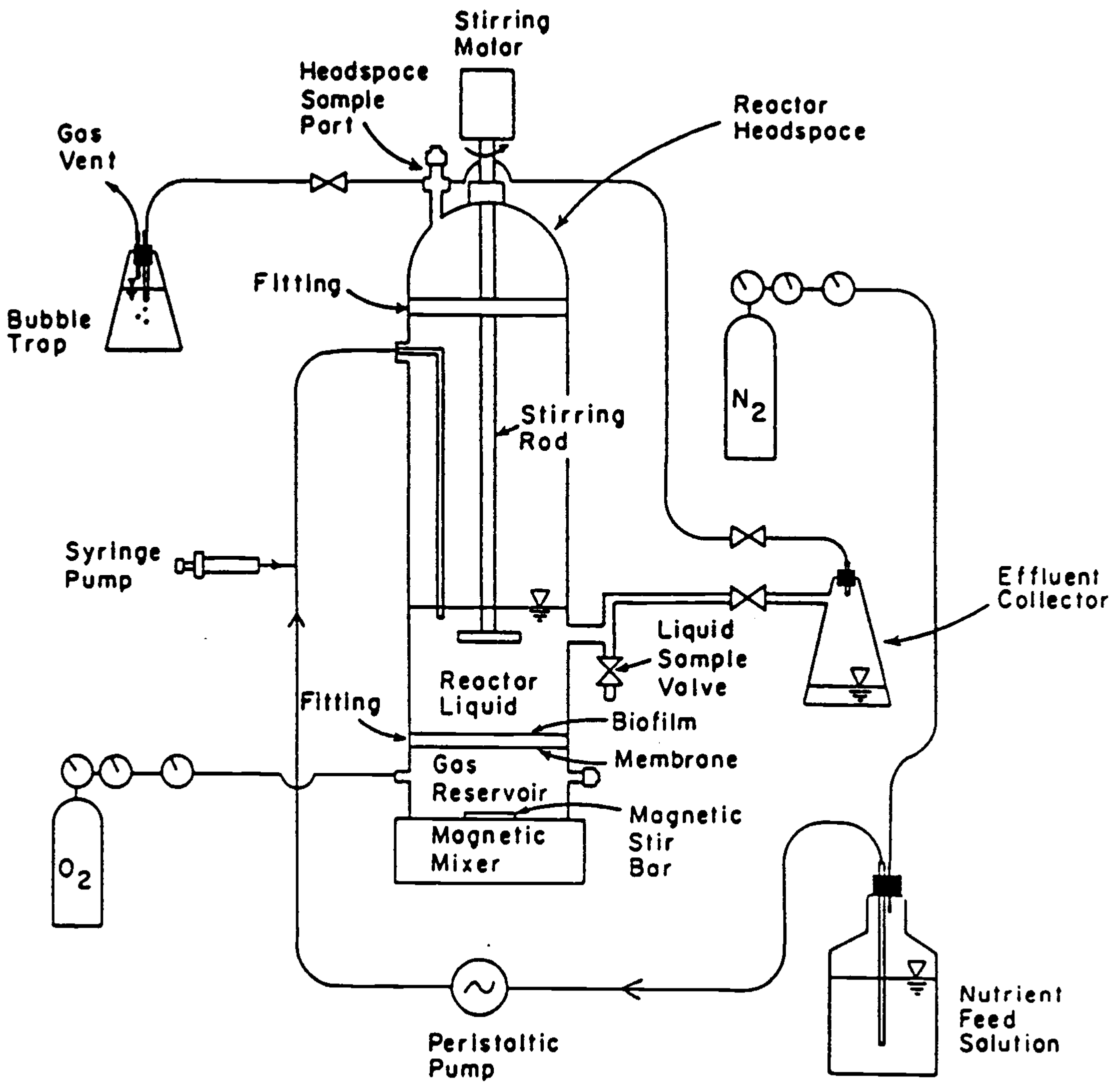


Figure 2. GPMS biofilm reactor design schematic

using a peristaltic pump and Teflon tubing (Cole-Parmer Instrument Company, Chicago, IL). During the initial 101 days of the experiment, the reactor was fed 50 ml/day and had an average liquid volume of 500 ml, resulting in an average hydraulic retention time of 10 days. From day 102 to day 220, the feed rate averaged 122 $\pm$ 6 ml/day and the average reactor liquid volume was 710 ml, resulting in an average hydraulic retention time of 5.8 days.

The nutrient feed consisted of mineral salts, trace elements, vitamins and carbon sources necessary to sustain cell growth for both aerobic and anaerobic microorganisms (Table 1). The mineral salts, trace elements and vitamins used were recommended by Owen et al. (1979). Nickel chloride, iron chloride, and sodium sulfide were added to the feed solution to enhance anaerobic metabolism (Shelton and Tiedje, 1984; Speece, 1983). For the first 126 days of flow to the reactor, the concentration of mineral salts and trace elements was almost double that of Table 1. The concentration was reduced at day 126 to eliminate precipitation of solids in the feed tubing. A phosphate-carbonate buffer was used to maintain the pH between 6.6 and 7.0. Glucose, methanol, acetic acid and acetone were provided as sources of carbon and electron donors. Glucose, methanol and acetic acid were added directly to the nutrient feed solution, while acetone was used as a solvent for the PCBs and was injected separately.

The PCBs used in this experiment (Figure 1) were obtained as solids (Ultrascientific, Hope, RI) and subsequently dissolved in reagent grade acetone to make the initial PCB stock solution (Table 2). This solution was diluted two or ten times, depending on the desired influent feed concentration. The diluted PCB stock solution was added

Table 1. Nutrient Feed Solution

Mineral Salts & Trace Elements Solution

compound	g/l-stock[a]	mg/l-feed	mmol/l-feed
$CaCl_2 \cdot H_2O$	16.7	125.25	0.85
NH_4Cl	26.6	199.50	3.73
$MgCl_2 \cdot 6H_2O$	120.0	900.00	4.43
$MnCl_2 \cdot 4H_2O$	1.3	9.75	0.05
KCl	86.7	650.25	8.72
$CoCl_2 \cdot 6H_2O$	2.0	15.00	0.06
H_3BO_3	0.38	2.85	0.05
$CuCl_2 \cdot 2H_2O$	0.18	1.35	0.01
$Na_2MoO_4 \cdot 2H_2O$	0.17	1.28	0.005
$ZnCl_2$	0.14	1.05	0.077
$FeCl_2 \cdot 4H_2O$[b]	0.19	1.43	0.007
$NiCl_2 \cdot 6H_2O$[b]	0.003	0.02	0.0001
$Na_2S \cdot 9H_2O$[b]	16.1[c]	2.42	0.01

Vitamin Solution

compound	g/l-stock[a]	mg/l-feed
biotin	0.02	0.02
folic acid	0.02	0.02
pyridoxinehydrochloride	0.1	0.1
riboflavin	0.05	0.05
thiamin	0.05	0.05
nicotinic acid	0.05	0.05
pantothenic acid	0.05	0.05
B_{12}	0.001	0.001
p-aminobenzoic acid	0.05	0.05
thiotic acid	0.05	0.05

COD Source

compound	g/l-feed	mg COD/l-feed
glucose	0.40	428
methanol	0.63	948
acetic acid	0.84	895
acetone	0.33	729

Buffer Solution

compound	g/l-feed
Na_2HPO_4	5.13
$NaH_2PO_4 \cdot H_2O$	1.89
$NaHCO_3$	0.55

[a] after the media described by Owen et al. (1979)
[b] addition made to Owen et al.'s media
[c] sodium sulfide was added separately to nutrient solution

to the nutrient feed line just before entrance of the Teflon tubing into the reactor. A 500-ul Hamilton gas-tight syringe was used with a syringe pump (Sage Instruments, Cambridge, MA) to inject the PCB/acetone solution at a rate of 50 ul/day. The end of the Teflon influent line was immersed 1 cm beneath the surface of the reactor liquid to reduce air stripping of PCBs.

There were four distinct periods in reactor influent conditions (Table 3). During Period 1 (the biofilm acclimation period), the reactor was run at a hydraulic retention time of 10 days and fed PCBs at the initial high concentration (Table 2). The initial PCB influent concentration of 110 ug/l was selected to exceed the aqueous solubilities of these compounds to compensate for PCB adsorption to the biofilm, reactor and tubing surfaces. During Period 2, the PCB influent concentration remained the same as in Period 1, but the hydraulic retention time was lowered to 5.8 days. During Periods 3 and 4 the hydraulic retention time remained at 5.8 days. A step decrease of the influent PCB concentration to 21 ug/l for each PCB was used during Period 3, and a step increase to 110 ug/l was used during Period 4.

The headspace above the liquid was initially purged with nitrogen. Pressure created by gas production due to bacterial metabolism was vented through a bubble trap connected to the headspace sample port. The bubble trap consisted of a 250-ml Erlenmeyer flask filled with 200 ml of a 5% sulfuric acid-saturated sodium chloride solution. The acid-salt solution restricted the flow of gases through the bubble trap, thus maintaining an anaerobic reactor headspace. In order to produce a steady liquid flowrate, it was necessary that the reactor and effluent

Table 2. PCB Stock and Feed Concentrations

Compound	Initial Acetone Stock Solution (mg/ml)	Reactor Influent, High (ug/l)	Reactor Influent, Low (ug/l)
2,3′,4,4′-CB*	0.52	110	21
2,3,4,5,6-CB	0.50	110	21
2,2′,4,4′,6,6′-CB	0.53	110	21
2,2′,3,4,4′,5′,6-CB	0.54	120	22
2,2′,3,3′,4,4′,5,5′-CB	0.52	110	21

* CB = chlorobiphenyl

Table 3. Reactor Feed Periods

Period	Time (days)	Hydraulic Retention Time (days)	PCB Feed Concentration
1	0-102	10.0	high
2	102-122	5.8	high
3	122-209	5.8	low
4	209-220	5.8	high

collector headspace pressures were equal. This was achieved by connecting the reactor and effluent collector headspaces with tygon tubing.

Analytical Methods

Liquid Sampling Method

The removal of a liquid sample from the reactor was achieved by following several distinct steps. First, the effluent collector and bubble trap lines were clamped shut. A nitrogen-filled balloon was attached to the headspace sample port of the reactor to replace the liquid volume removed with nitrogen. The liquid was drained by opening the Teflon buret valve on the effluent arm. Ten ml of liquid were wasted before collecting a 30 ml sample directly in a Teflon centrifuge tube.

PCB Analysis Procedure

Samples for gas chromatograph-electron capture detection (GC/ECD) analyses, as well as for gas chromatograph-mass spectrometry (GC/MS) analyses were prepared as follows:

1. 30 ml liquid samples were collected in Teflon centrifuge tubes.
2. The samples were centrifuged 10 minutes at 10000 rpm.
3. Using a glass pipet, 20 ml of sample supernatants were transferred to 25-ml glass vials with Teflon lined caps.
4. 50 ul of a 2.32 ng/ul tetrachloronaphthalene/hexane solution were added to samples to serve as an internal standard.
5. 2 ml of hexane were added to the samples.

6. The samples were shaken 4 minutes using a wrist action shaker.

7. The samples were centrifuged at 2500 rpm for 5 minutes to separate any emulsion that may have formed during extraction.

8. The hexane phase was transferred to a 2-ml amber auto-sampler vial using pasteur pipets, and stored at $4^{o}C$.

Biofilm Sampling Procedure

Solids were removed from the biofilm using a 30 ml glass syringe. Twenty-five cm of (3 mm diameter) Teflon tubing attached to the syringe was used to carefully remove approximately 0.5 grams of solids in 4 ml of reactor liquid from the surface of the reactor biofilm. The following procedure was followed to extract PCBs and PCB-metabolites from the solid phases (Goerlitz and Law, 1974):

1. Solids (and some liquid) were placed in a 4-ml amber glass vial.

2. Three small scoops of copper pellets were added to remove sulfur compounds often present in bacterial solids.

3. 1 ml of reagent grade acetone was added to the samples.

4. The samples were shaken for 20 minutes using a wrist action shaker.

5. 50 ul of the tetrachloronaphthalene internal standard were added to the samples.

6. 2 ml of hexane were added to the samples.

7. The samples were shaken for 10 minutes using a wrist action shaker.

8. The samples were centrifuged 10 minutes at approximately 2500 rpm.

9. The hexane phases of the samples were transferred to 25-ml glass vials using pasteur pipets.

10. The samples were washed two times by adding 10 ml of distilled water and gently rolling the samples.

11. The hexane phases were transferred to 2-ml amber auto sampler vials using pasteur pipets, and stored at $4^{o}C$.

Gas Chromatograph Procedure

The gas chromatographic analyses were performed using a splitless injection system with a Hewlett Packard (HP) 5890 GC (Avondale, PA) and HP7673A auto-sampler. An HP3393A integrator recorded the signal from the electron capture detector. A 30 m x 0.33 mm DB-5 (J&W Scientific, Deerfield, IL) fused silica capillary column with a 0.25 um film thickness was used for separating compounds. Helium was supplied as the carrier gas at a column head pressure of 5 psi. A makeup gas of 95% argon/5% methane was supplied to the injection port at a rate of 25 ml/min.

The injection and detection port temperatures were 300°C. The initial oven temperature was held at 45°C for 2 minutes, then raised to 200°C at a rate of 20 ^{o}/minute. The oven was held at 200°C for 15 minutes, and at a rate of 4 ^{o}/minute, raised to 245°C where it was held for 27 minutes, resulting in a total run time of 63 minutes.

Unidentified peaks were determined by gas chromatography with mass spectrometry (GC/MS). GC/MS analyses were performed at the EPA laboratory at the Mark O. Hatfield Marine Sciences Center in Newport, Oregon. An HP5890 GC and an HP5988 MS were used with a capillary column similar to the column used for GC/ECD analysis. Carrier gas was supplied at a column head pressure of 20 psi. The injector port temperature was 300°C, the interface was 280°C, and the source was 200°C. The same temperature program that was used for GC/ECD analysis was used with GC/MS analysis.

COD Sample Method

Chemical oxygen demand (COD) samples were obtained from the supernatant of the centrifuged liquid samples. Five ml of the sample supernatant were transferred to a 25 ml glass vial and diluted to 25 ml with distilled water. Three drops of concentrated sulfuric acid were added to the sample before storage at 4°C. Samples were analyzed for COD content using the Closed Ampule Method (Standard Method #508C, APHA, 1985).

Headspace Samples

Headspace samples were taken periodically by removing a 100-ul gas sample from the headspace sample port using a 250-ul gas-tight syringe. The gas samples were injected into a Fisher Model 25V gas partitioner (Fisher Scientific Co., Pittsburgh, PA) which was equipped with a 6.5 ft long x 3/16 inch diameter column packed with 42-60 mesh Molecular Sieve 13X to separate nitrogen, oxygen and methane gas, and a 30 inch x 1/4 inch column packed with 30% HMPA on Columpak (60 to 80 mesh) to separate carbon dioxide. Helium, supplied at a pressure of 10 psi, was used as the carrier gas. A thermal conductivity cell within the gas partitioner was used to detect the gases.

RESULTS

Reactor operation was monitored by measuring liquid influent and effluent chemical oxygen demand (COD), by taking reactor headspace gas samples, and by extracting liquid and biofilm samples. COD samples were taken each week to measure the relative bacterial activity in the reactor. Headspace samples were taken a minimum of once weekly to check qualitatively for methane. Liquid samples were taken to monitor concentrations of the parent compounds and to check for PCB metabolites, from a maximum of three times a day to a minimum of once per five days, depending on the experimental program.

Because the first 102 days of continuous feed to the reactor were primarily to acclimate the biofilm to PCBs, and the following 20 days were to develop steady state in the reactor at a hydraulic retention time of 5.8 days, results will be presented for the period in which the only changes in reactor conditions were the PCB feed concentrations, unless otherwise mentioned. This is during the period between day 120 and day 220.

COD Results

At day 120, the reactor had been fed at a level of 3200 mg/l COD for 43 days (Figure 3). Following the decrease in mineral salts and trace elements in the feed on day 127, the effluent COD rose to an average of 1600 mg/l which corresponded to 50% removal of the influent COD. It remained around 1600 mg/l for forty days until the biofilm was disrupted at day 165. The biofilm disruption was due to the withdrawal of a small sample of the biofilm. By day 180, almost three hydraulic

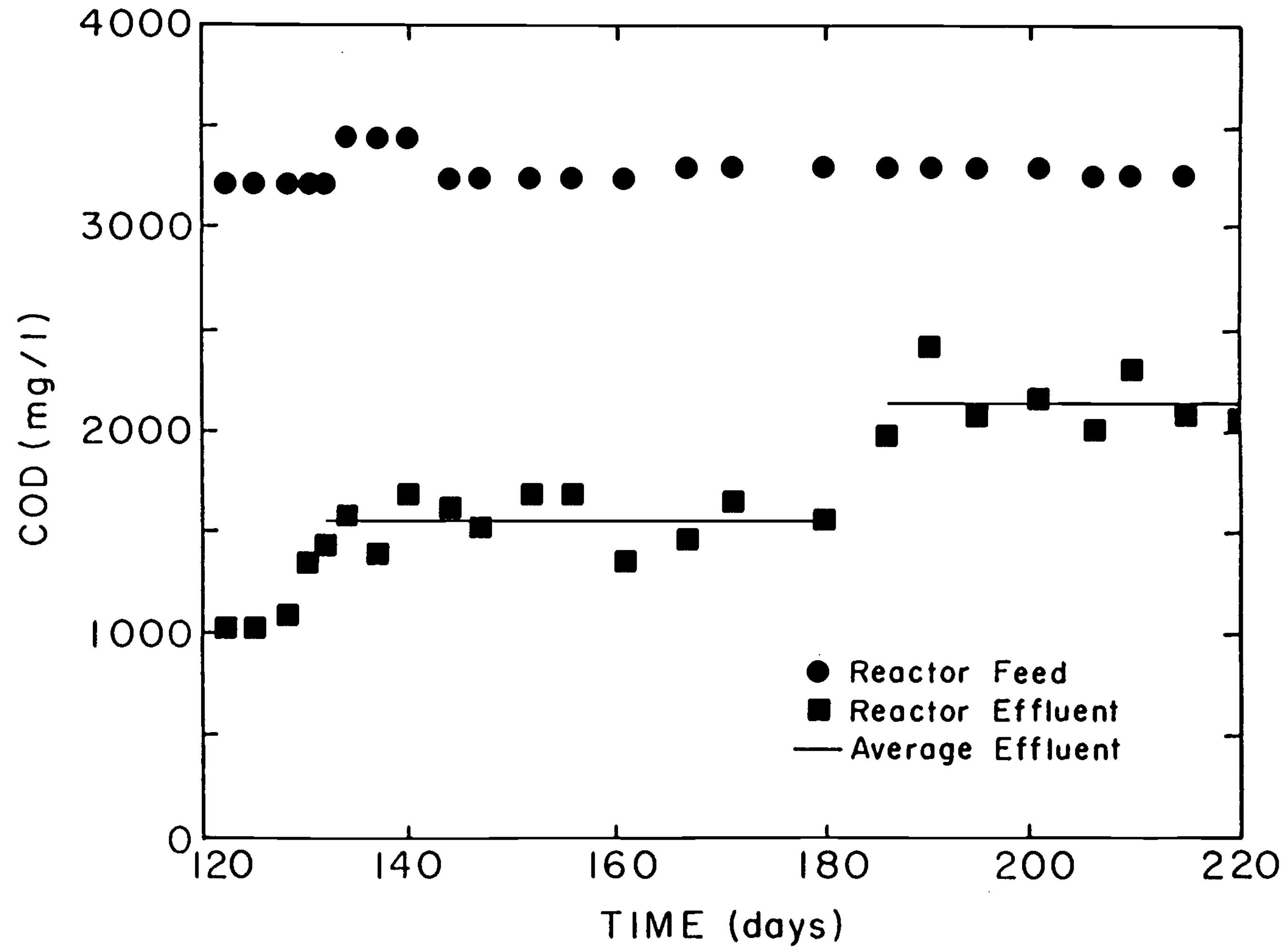


Figure 3. Chemical oxygen demand in reactor liquid

retention times after the disruption, the reactor liquid reached a new steady state at 2100 mg/l COD (34% removal of influent COD). The reactor liquid COD remained at this level for the duration of the experiment. The two stages of steady state with regard to COD are shown as solid lines in Figure 3.

Anaerobic Results

Initially, only nitrogen, carbon dioxide and oxygen were present in the reactor headspace, with the major component being nitrogen. Methane first appeared in the headspace samples at day 45, shortly after sodium sulfide was added to the feed solution. For the period from day 120 to day 220, the headspace averaged (by volume) 82.8% nitrogen, 10.7% carbon dioxide, 4.7% oxygen, and 1.9% methane.

PCB Concentrations in Reactor Liquid

The PCBs were fed to the reactor at a concentration of approximately 110 ug/l each (Table 2) for the first 123 days of the experiment. At day 123, the PCB concentration in the feed was decreased by a factor of five to about 21 ug/l each. This step decrease in PCB influent concentration resulted in a decrease in the concentration of each PCB in the reactor effluent (Figures 4 through 8). By day 150, tetra-, penta-, hexa- and heptachlorobiphenyl (hepta-CB) reached a steady state concentration around 2 to 3 ug/l, and octa-CB was present at 0.5 to 1 ug/l. A sharp rise of effluent concentrations for the parent compounds occurred after the biofilm sample taken on day 165. As mentioned before, the biofilm sample

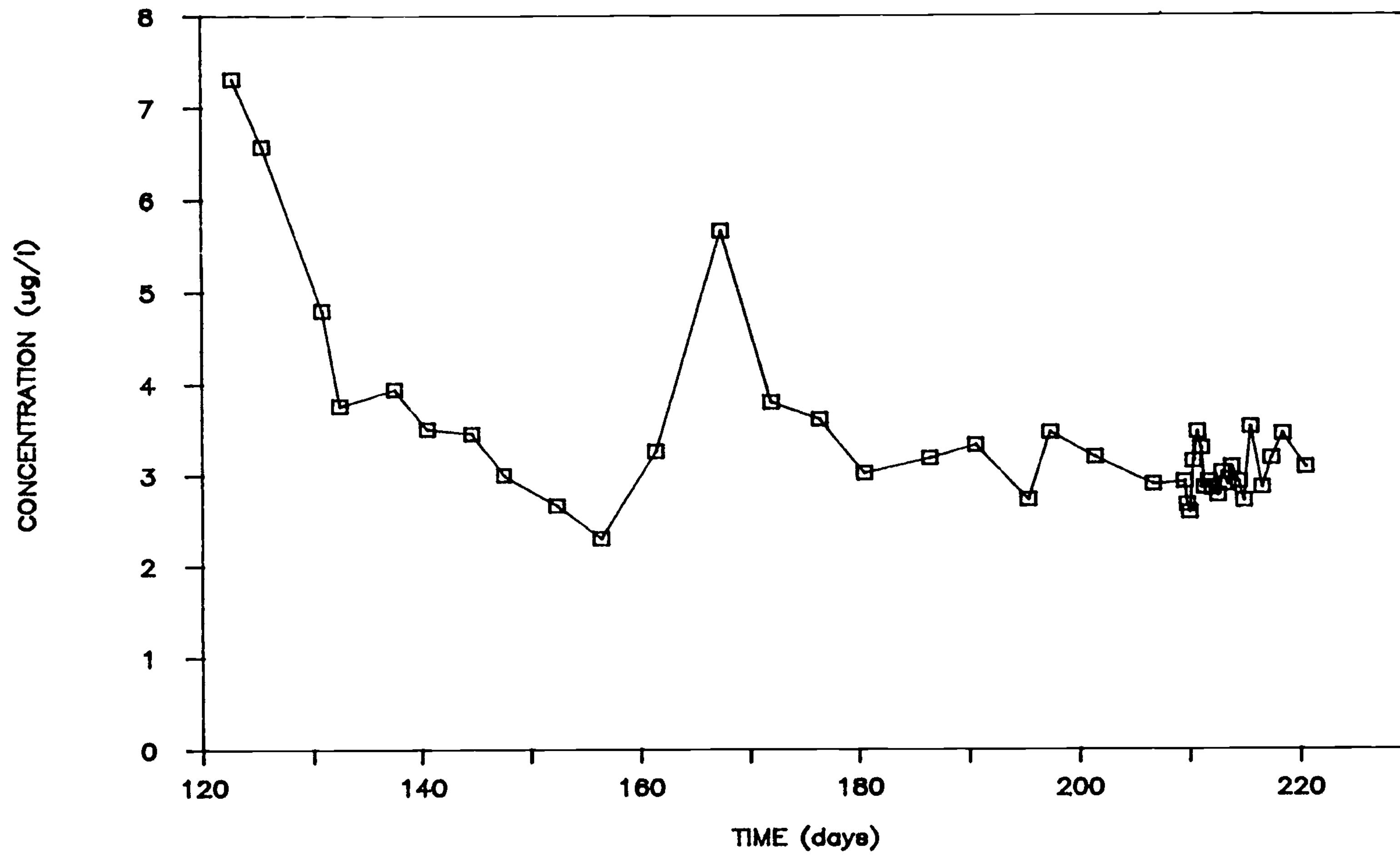


Figure 4. Concentration of 2,3'4,4'-CB in reactor effluent

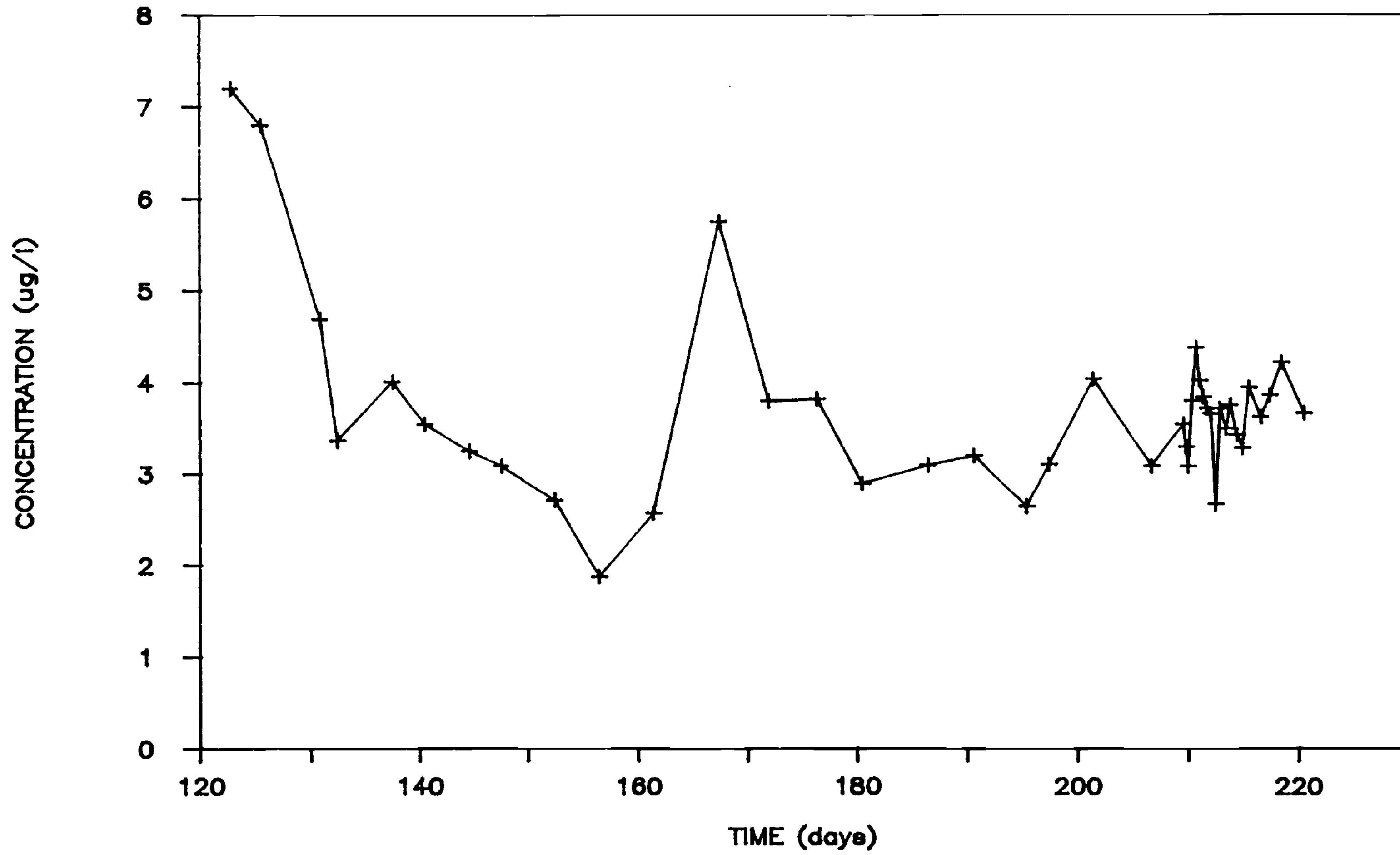


Figure 5. Concentration of 2,3,4,5,6-CB in reactor effluent

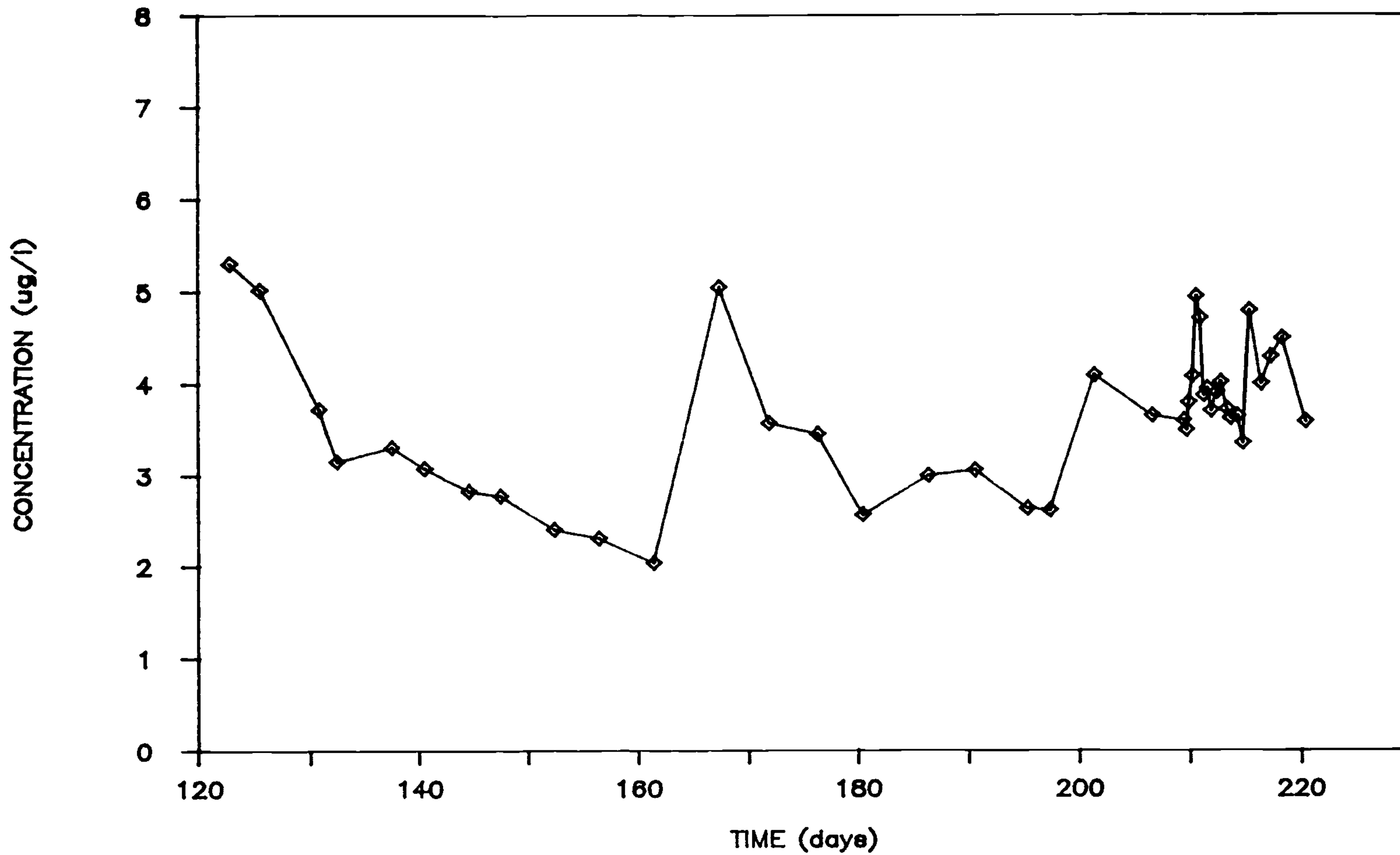


Figure 6. Concentration of 2,2'4,4'6,6'-CB in reactor effluent

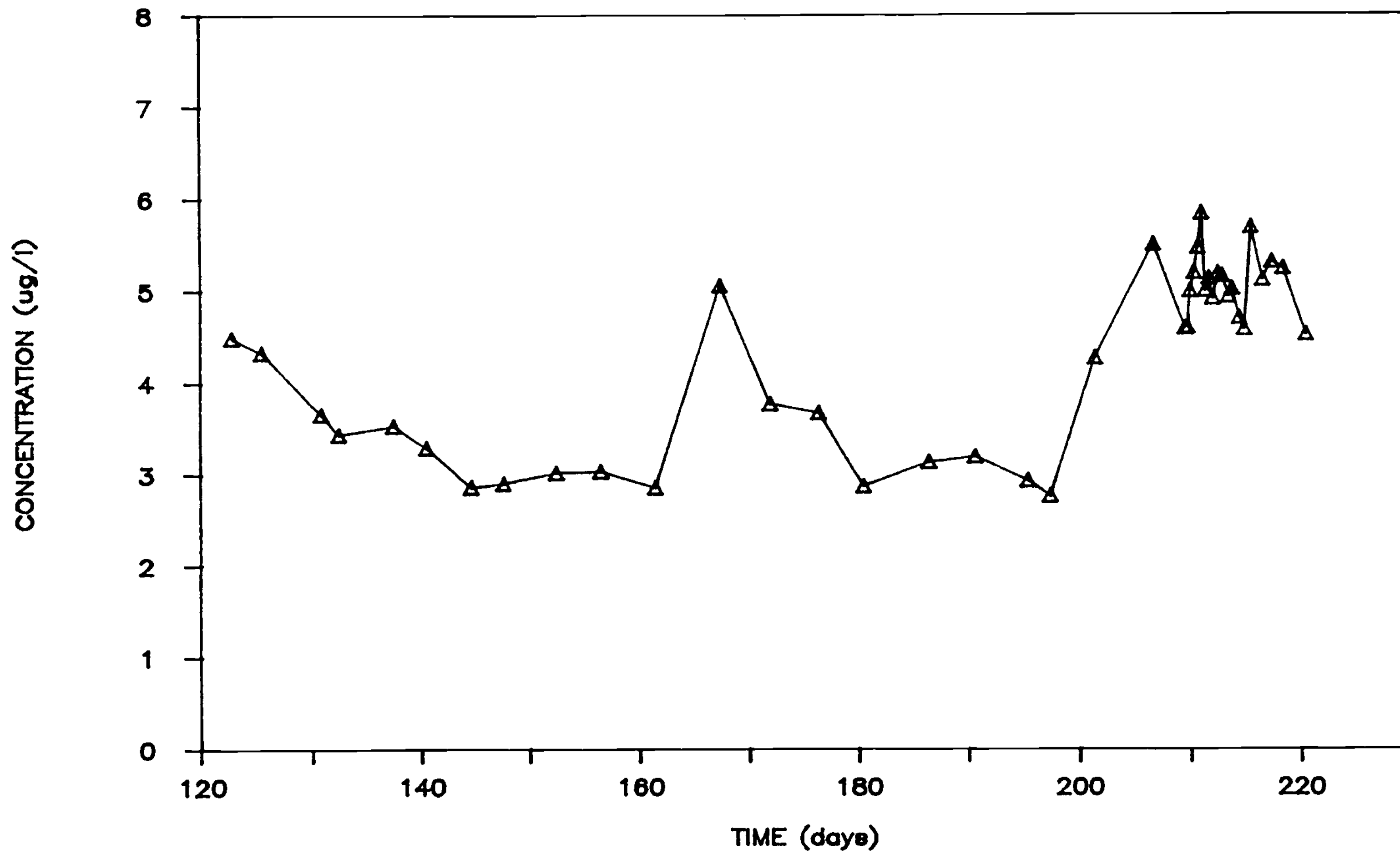


Figure 7. Concentration of 2,2'3,4,4'5'6-CB in reactor effluent

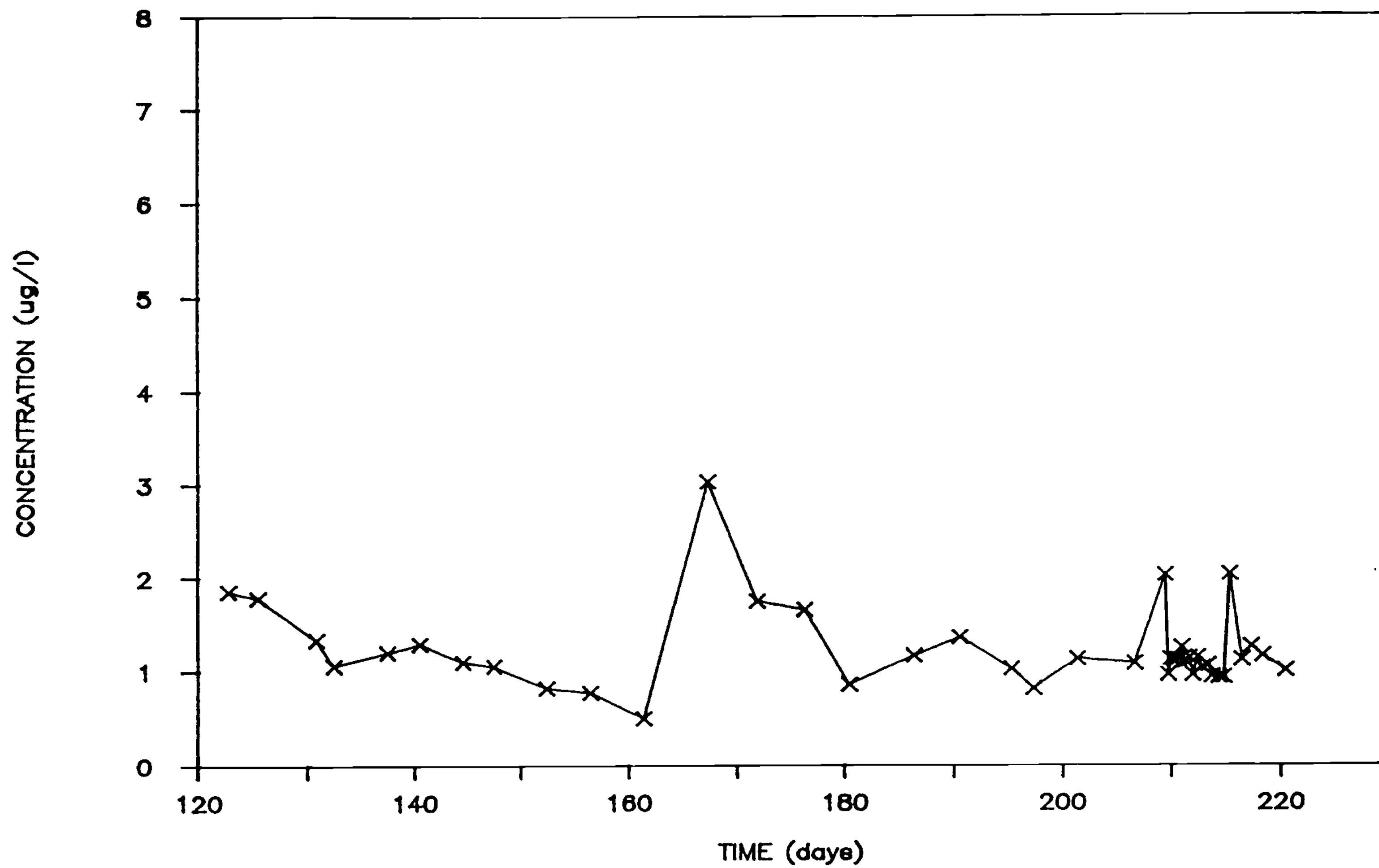


Figure 8. Concentration of 2,2′3,3′4,4′5,5′-CB in reactor effluent

disrupted the reactor steady state. By day 181, PCB effluent concentrations approached the levels before the disturbance.

The second step change in PCB influent concentrations at day 209 resulted in a slight increase in the effluent concentration of penta-, hexa-, and hepta-CB, while tetra- and octa-CB concentrations changed very little.

PCB Removal

During the initial period before the step changes were made to the PCB influent concentrations, removal of PCBs from the reactor influent was in the range of 92 to 95% (Table 4). Following the step decrease in PCB influent concentrations at day 123, removal leveled out at 83 to 85% for tetra-, penta-, hexa- and hepta-CB, and at about 94% for octa-CB. After the step increase in PCB concentrations at day 209, treatment resulted in 95 to 97% removal of the four lesser chlorinated PCBs, while about 99% of the octa-CB was removed from the reactor liquid feed.

Metabolite Analysis

Because PCBs have a very low aqueous solubility and a high solids partition coefficient, their removal from the liquid phase by adsorption in a biological reactor is very high. The slow rate of degradation of PCBs and the analytical error involved in their measurement also makes it infeasible to determine biodegradation had occurred from PCB removal data. Thus, it is necessary to identify metabolic byproducts to prove that biodegradation of PCBs was

Table 4. Average PCB Percent Removal

Compound	Days 102-120	Days 120-209	Days 209-220
2,3'4,4'-CB	92.4	83.6	97.3
2,3,4,5,6-CB	91.8	83.8	96.7
2,2'4,4'6,6'-CB	93.7	85.1	96.4
2,2'3,4,4'5'6-CB	94.3	84.3	95.8
2,2'3,3'4,4'5,5'-CB	95.0	94.2	98.9

occurring. At least three factors may have hindered the accumulation of metabolites in the liquid phase: the slow degradation rate of PCBs, the possibility that PCB metabolites may have degraded faster than their parent compounds, and the sorption of metabolites to solids in the reactor system. Step changes in PCB influent concentration were made to evaluate whether potential metabolites were responding to changes in influent PCB concentrations.

On day 165 and at the end of the experiment, biofilm samples were taken to ascertain whether detectable amounts of metabolites had adsorbed to the solids. GC/MS analyses on liquid and solid samples were performed to further identify potential metabolites.

To determine if metabolites were occurring in reactor liquid and biofilm samples, GC/ECD chromatograms were compared to GC/ECD chromatograms of reactor influent extraction samples (Figure 9). By comparing these chromatograms, it was possible to identify new peaks in the liquid and biofilm extraction sample chromatograms which may represent the occurrence of metabolites. The sample volume for the influent and effluent samples in Figure 9 were identical and the samples were extracted using the same procedure (note the similar internal standard areas for influent and effluent samples). Based on the decrease in peak area for each of the parent compounds on day 220 (Figure 9), the degree of PCB removal is visually apparent. Possibly due to the effect of sorption and slow degradation kinetics, liquid samples did not result in any definite signs of metabolite formation. However, the GC/ECD chromatogram of the biofilm solids sample taken at day 220 revealed the presence of Peak B which occurred 0.2 minutes prior to the internal standard, Peak C (Figure 10). This chromatogram

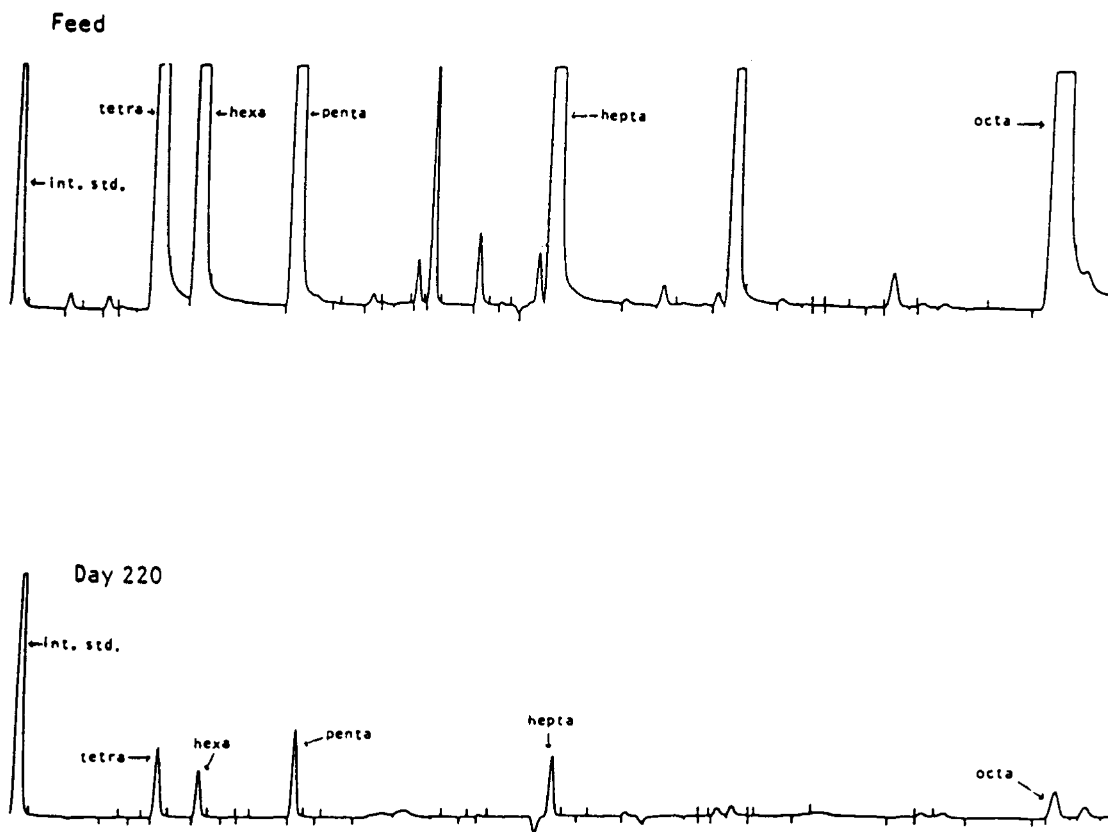


Figure 9. GC/ECD chromatograms of reactor feed and reactor liquid sample taken at day 220

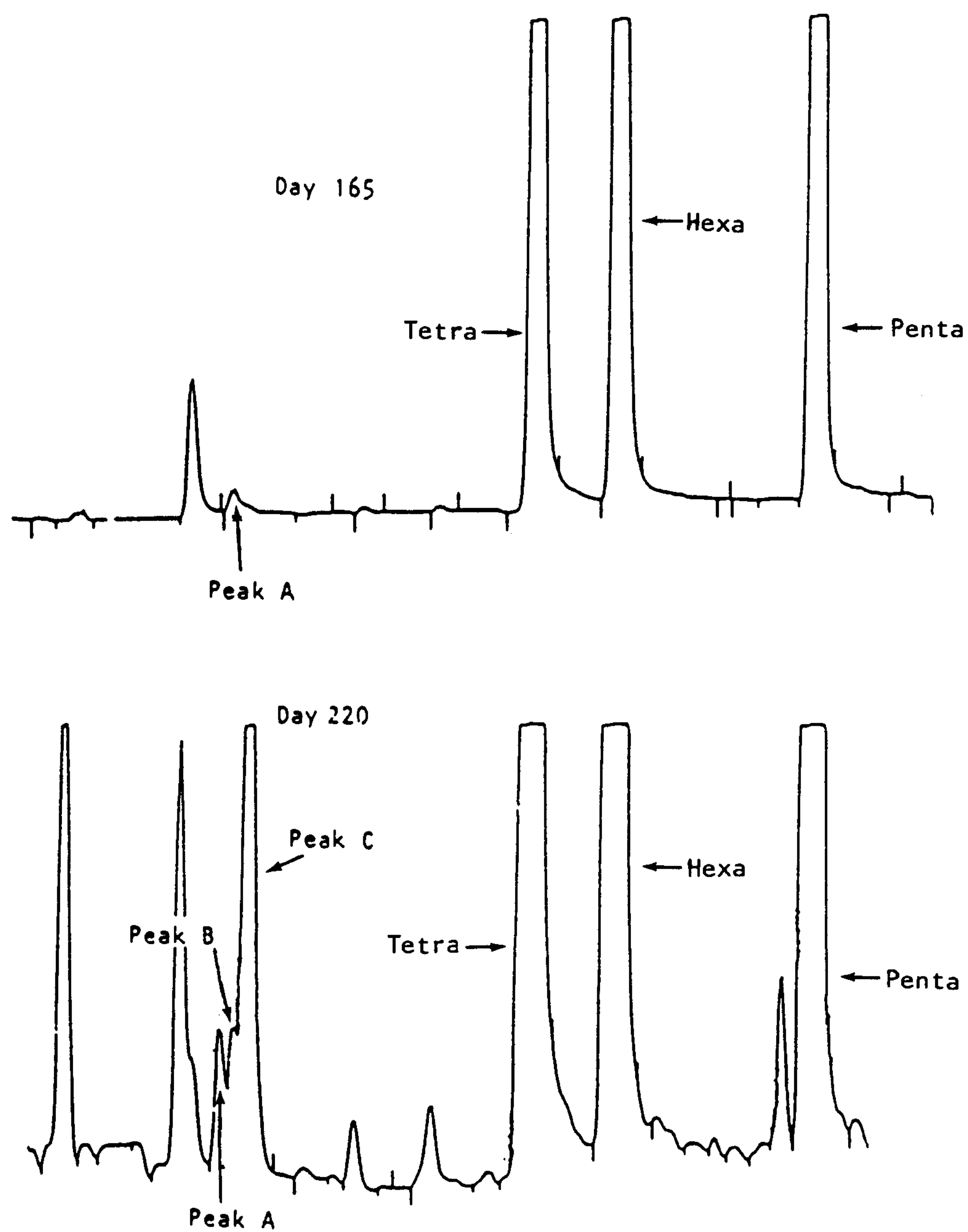
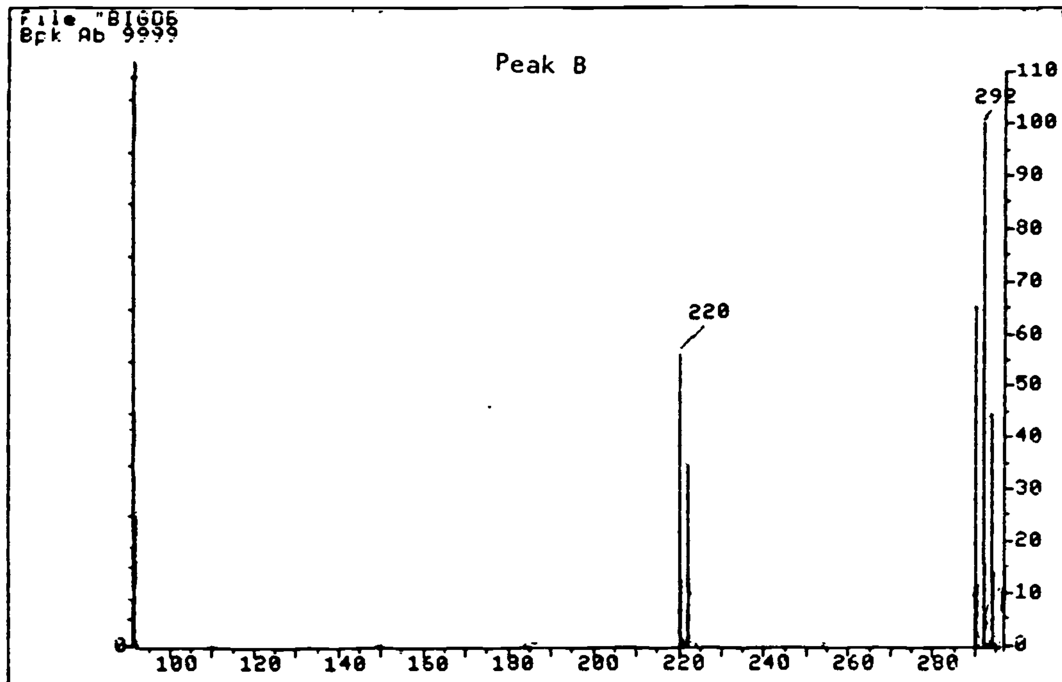


Figure 10. GC/ECD chromatograms of biofilm samples taken on day 165 and day 220

was compared to the chromatogram of the biofilm extraction from the sample taken at day 165. The internal standard was not included in the sample taken on day 165 to be sure Peak B was not being covered by the internal standard. In standards and blank extractions which included the internal standard, Peak B was not present. It can be seen in Figure 10 that the presence of Peak B developed between day 165 and day 220, while Peak A is present in both samples. The relative retention time of Peak B was identical to the relative retention time of 2,3,5,6-CB. Subsequent GC/MS analyses of the biofilm sample taken on day 220 revealed the presence of the five major ions of 2,3,5,6-CB (Figure 11). These five ions occurred at a retention time which matched the relative retention time of Peak B in the GC/ECD chromatogram of the day 220-biofilm sample. The similarity between the mass ratios of the five major ions for Peak B and 2,3,5,6-CB is shown in Figure 11. The maximum abundance for the Peak B mass spectrum was 23, which was too low for the mass spectrum to be computer-generated. Thus, the mass ion peaks for Peak B in Figure 11 were drawn manually.

Similarly, several ions were identified as mono- and dihydroxylated PCBs in some of the reactor liquid samples, however the concentrations were too low to make absolute identifications possible.



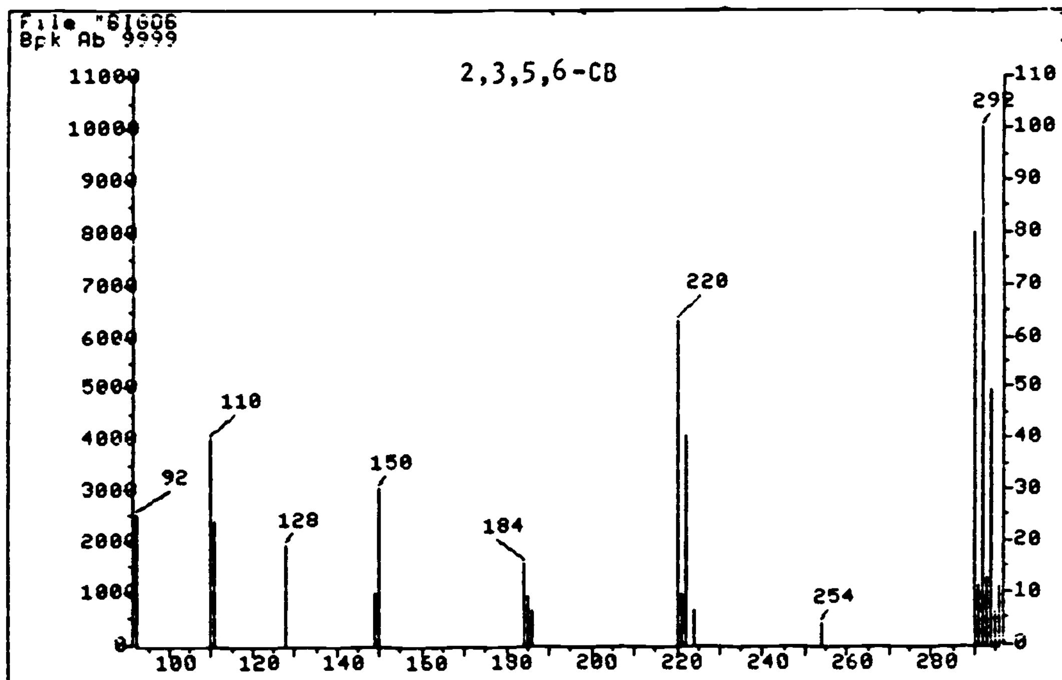


Figure 11. Mass spectrums of Peak B and 2,3,5,6-CB

DISCUSSION

Steady State

It is evident from COD data that two general periods of steady state were achieved during the period between days 120 and 220. The first period occurred from day 140 to 180. During this time 50% of the influent COD was being consumed. The second steady state period occurred after the biofilm reequilibrated from the disruption caused during the biofilm sampling. A likely explanation for the decrease in %COD removal during this second steady state period is that there were fewer active microorganisms in the biofilm following the solids sample taken on day 165.

Anaerobic Discussion

The appearance of methane on day 43 shortly after introduction of sodium sulfide (which is a reducing agent) to the feed suggests that sulfide enhanced conditions for anaerobic growth.

PCB Concentrations in the Reactor Liquid

On day 123 the PCB influent concentrations were reduced 83%. The reactor liquid concentrations of each parent PCB decreased following this decrease in influent concentrations, however, the concentrations decreased considerably less than the 83% decrease in PCB influent concentrations. This may have been due to oversaturation of the PCBs in the reactor at the initial PCB influent concentrations, such that by decreasing the influent concentrations five times merely brought some

or all of the parent compounds below their solubility limits. Another possibility is that reaction kinetics and sorption rates are functions of concentration such that lower influent concentrations resulted in slower degradation.

The sharp rise in PCB concentrations following the biofilm disturbance may have been due to the increased amounts of PCB-saturated suspended and colloidal solids in the reactor liquid. If a fraction of these solids were not removed from the liquid phase during centrifugation and were extracted during the liquid sample procedure, then the PCB mass extracted would have increased considerably.

There was a slight increase in the penta-, hexa-, and hepta-CBs' effluent concentrations following the step increase in PCB influent concentrations on day 209. The tetra- and octa-CBs had negligible changes in concentration following this increase in influent concentrations. The cause for this less than expected increase may have been due to the solution being saturated with PCBs. A related effect may have been that the PCBs were forming a separate phase by precipitating on the surfaces of the reactor vessel, liquid and biofilm. Another possibility is that the time required for the increase in influent concentrations to have an impact on the effluent concentrations was longer than the eleven days (two hydraulic retention times) in which samples were taken.

PCB Removal

Biodegradation products were not detected in large amounts even though PCB removal efficiencies were very high. The occurrence of adsorption and the slow degradation rates may have been the main

factors resulting in nondetectable concentrations of metabolites in the liquid and biofilm samples. Thus it appears that the major removal pathway involved adsorption of PCBs to microorganisms in the reactor biofilm. Because bacteria were continually growing, new sites for PCB sorption were continually appearing. Furthermore, PCBs were being removed from the reactor liquid when PCB-saturated cells decayed and flowed out with the reactor effluent.

Metabolite Identification

The occurrence of the peak which corresponds with 2,3,5,6-CB in the biofilm sample taken at day 220 suggests that anaerobic degradation may have been occurring in the GPMS biofilm reactor. The only parent compound capable of being dechlorinated to 2,3,5,6-CB was 2,3,4,5,6-CB. This would have occurred by removal of the para-chlorine from the 2,3,4,5,6-CB. A similar reductive dechlorination of 2,3,4,5,6-CB to 2,3,5,6-CB was observed by Low and Woods (1988) and Tiedje et al. (1987). This similar dechlorination pattern suggests that removal of the para-chlorine of 2,3,4,5,6-CB may be the first step taken by many anaerobic populations in degrading PCBs which are chlorinated at each site on one ring. Because the biofilm had been exposed to PCBs for less than eight months, it is possible that after a longer period of time a proficient PCB-acclimated anaerobic population would exhibit even more evidence of PCB dechlorination.

The lack of confirmed hydroxylated PCBs in the reactor liquid and biofilm in GC/ECD analysis may be explained in part by the relatively poor partitioning of hydroxylated compounds in hexane during the hexane extraction procedures. Because the aerobic metabolites would be

present in low concentrations to begin with, and because sorption would remove a high percentage (from liquid samples) as well, the poor extractability of hydroxylated compounds resulted in only circumstantial proof of the presence of hydroxylated metabolites following GC/MS analyses.

SUMMARY & CONCLUSIONS

Steady State

As a result of COD data gathered from this experiment, it is evident that maintaining a relatively constant mass of microorganisms in the GPMS biofilm reactor over a period of at least forty days is possible. This was accomplished by maintaining the reactor conditions constant over the given time period. It was shown that perturbations in feed content such as reducing mineral salts and trace elements altered the COD consumption considerably. The biomass present in the biofilm was also a factor in determining the effluent COD, as shown by the rise in reactor effluent COD following the biofilm sample taken on day 165.

Anaerobic & Aerobic Growth

Sodium sulfide enhanced anaerobic growth in the reactor. This may have been due to its ability to provide a reducing environment in the bulk liquid. Before the addition of sodium sulfide, methane production was not detected.

PCB Removal

The continuous treatment of a PCB contaminated water using the GPMS biofilm reactor yielded removal of PCBs from 83 to 99 percent, depending on PCB influent concentrations and the degree of PCB chlorination. The major PCB removal pathway involved adsorption of PCBs to solids in the reactor. However, the occurrence of a peak which corresponded to 2,3,5,6-CB in the GC/ECD chromatogram of the final

biofilm sample suggests reductive dechlorination of 2,3,4,5,6-CB may have been occurring. GC/MS analyses further suggested this by identification of the five major ions of 2,3,5,6-CB at the same relative retention time as the potential metabolic product. Chlorinated hydroxybiphenyls were only tentatively identified, as their concentrations were too low to make positive identifications possible.

Conclusions

1. By maintaining a constant influent solution and flow rate, and avoiding biofilm disturbances, a continuous flow GPMS biofilm reactor may be operated at steady state with respect to COD removal.

2. By supplying oxygen as the supporting gas and by keeping the headspace closed to the atmosphere, it is possible to maintain the simultaneous growth of anaerobic and aerobic microorganisms on a gas-permeable membrane.

3. Treatment of PCBs using a GPMS biofilm reactor results in high removal efficiencies from the liquid phase. The primary removal pathway is adsorption to biomass in the reactor.

4. Biofilm extraction data suggests that reductive dechlorination of 2,3,4,5,6-CB to 2,3,5,6-CB occurred in the GPMS biofilm reactor.

CHAPTER TWO

SIGNIFICANCE & RECOMMENDATIONS

Presently, PCB treatment consists primarily of landfill storage and incineration. Landfill storage is merely a temporary solution to wastes which continue to pose a threat to the environment in the event of containment leakage. Incineration is a good treatment method in that it is a destructive process which may result in the complete elimination of PCB, however, there is still the chance of emission of toxic byproducts due to incomplete PCB combustion.

It has been demonstrated in research projects that several species of bacteria are capable of aerobically degrading PCBs. Other studies have revealed the potential for anaerobic degradation of PCBs by acclimated anaerobic bacteria. The use of these two groups of bacteria in a combined aerobic/anaerobic GPMS biofilm reactor could be an effective treatment process to reduce the toxic effects of PCBs in contaminated waters.

The maintenance of a GPMS biofilm reactor process would require supplying sufficient nutrients and buffer capacity to develop and sustain a healthy biofilm, along with careful observation of reactor conditions such as pH and temperature, not unlike what is done at present municipal and industrial biological treatment operations.

The experiment described in this report has shown the feasibility of operating a GPMS biofilm reactor as a continuous feed process, however, these reactors could be adapted to run as batch processes as well. A batch process would provide a longer contact time for more complete degradation of chlorinated aromatic compounds.

Recommendations

Reactor Design

To increase interaction between toxic compounds in the contaminated water and microorganisms in the biofilm, it would be advantageous to design the reactor such that there is a higher biofilm surface area to liquid volume ratio. This could be accomplished by reducing the reactor liquid volume, and/or by designing a new system which has a larger membrane surface area.

Experimental Procedure

By altering the liquid and biofilm sampling procedures it may be possible to improve extraction results. To obtain highly adsorptive compounds without taking more biofilm samples, it may suffice to extract liquid samples that have not been centrifuged. In this way, colloidal and suspended solids in the reactor liquid can be extracted such that detectable amounts of metabolites may be observed in GC/ECD and GC/MS analysis. A solids analysis should be performed on reactor liquid samples regularly if this procedure is used. If the reactor is run at a relatively steady state, the suspended solids concentration should remain stable.

Another possibility to be considered is the extraction of larger liquid volumes. Following the extraction, the sample could be concentrated to yield higher concentrations which may be detected by GC/ECD and GC/MS analysis.

The use of different organic solvents may improve the efficiency of metabolite extraction. Methylene chloride could be advantageous for

its ability to remove chlorinated compounds more effectively than hexane. Following extraction, the methylene chloride samples could be transferred to isooctane, as methylene chloride interferes considerably with electron capture detection. Ethyl acetate is another solvent that could be useful for its ability to more effectively dissolve hydroxylated compounds, while at the same time being comparable to hexane in its ability to dissolve chlorinated aromatics.

LITERATURE CITED

1. Erickson, M. D., 1986. Analytical Chemistry of PCBs. Butterworth Publishers, Stoneham, MA.

2. Shelton, D. R., J. M. Tiedje. 1984. General Method for Determining Anaerobic Biodegradation Potential. Appl. Environ. Microbiol. 47:850-857.

3. Liu, D. 1982. Assessment of Continuous Biodegradation of Commercial PCB Formulations. Bull. Environm. Contam.Toxicol. 29:200-207.

4. Bailey, R. E., S. J. Gonslor, W. L. Rhinehart. 1983. Biodegradation of the Monochlorobiphenyls and Biphenyl in River Water. Environ. Sci. Technol. 17:617-620.

5. Bedard, D. L., R. Unterman, L. H. Bopp, M. J. Brennan, M. L. Haberl, C. Johnson. 1986. Rapid Assay for Screening and Characterizing Microorganisms for the Ability to Degrade Polychlorinated Biphenyls. Appl. Environ. Microbiol. 51:761-768.

6. Furukawa, K., N. Tomizuka, A. Kamibayashi. 1983. Metabolic Breakdown of Kaneclors (Polychlorobiphenyls) and Their Products by Acinetobacter sp. Appl. Environ. Microbiol. 46:140-145.

7. Bruggenman, W. A., J. van der Steen, D. Hutzinger. 1986. Reverse-Phase Thin-Layer Chromatography of Polynuclear Aromatic Hydrocarbons and Chlorinated Biphenyls Relationship with Hydrophobicity as Measured by Aqueous Solubility and Octanol-Water Partition Coefficients. J. Chromatogr. 238(2):335-346.

8. Yalkowsky, S. H., S. C. Valvani, D. MacKay. 1983. Estimation of the Aqueous Solubility of Some Aromatic Compounds. Residue Reviews. 85:43-55.

9. Bedard, D. L., R. E. Wagner, M. J. Brennan, M. L. Haberl, J. F. Brown. 1987. Extensive Degradation of Aroclors and Environmentally Transformed Polychlorinated Biphenyls by Alcaligenes eutrophus H850. Appl. Environ. Microbiol. 53:1094-1102.

10. Owen, W. F., O. C. Stuckey, J. B. Healy, Jr., L. Y. Young,P. L. McCarty. 1979. Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity. Water Research. 13:485-492.

11. Hutzinger, O., S. Safe, V. Zitko. 1974. The Chemistry of PCBs. CRC Press. Cleveland, OH. 269 p.

12. Brown, J. F., Jr., R. E. Wagner, H. Feng, D. L. Bedard, M. J. Brennan, J. C. Carnahan, R. J. May. Environmental Dechlorination of PCBs. To be published: Env. Toxicol. Chem.

13. Bedard, D. L., M. L. Haberl, R. J. May, M. J. Brennan. 1987. Evidence for Novel Mechanisms of Polychlorinated Biphenyl Metabolism in Alcaligenes eutrophus H850. Appl. Environ. Microbiol. 53:1103-1112.

14. Furukawa, K., N. Tomizuka, A. Kamibayashi. 1979. Effect of Chlorine Substitution on the Bacterial Metabolism of Various Polychlorinated Biphenyls. Appl. Environ. Microbiol. 38:301-310.

15. Masse, R., F. Messier, L. Peloquin, C. Ayotte, M. Sylvestre. 1984. Microbial Biodegradation of 4-Chlorobiphenyl, a Model Compound of Chlorinated Biphenyls. Appl. Environ. Microbiol. 47:947-951.

16. Tiedje, J. M., S. A. Boyd, J. F. Quensen. 1987. Reductive Dechlorination of PCBs in Anaerobic Microbial Communities. General Electric Company Research and Development Program for the Destruction of PCBs, Sixth Progress Report. Chpt 5:29-33.

17. Low, E. J., S. L. Woods. 1988. A Method for PCB Metabolite Determination in Anaerobic Fresh Water Sediments Using an Uplow Anaerobic Biofiltration Process (ANBIOF). In preparation.

18. Speece, R. E. 1983. Anaerobic Biotechnology for Industrial Wastewater Treatment. Environ. Sci. Technol. 17:416A-427A.

19. Mackay, D., S. Paterson, S. J. Eisenreich, M. S. Simmons. 1983. Physical Behavior of PCBs in The Great Lakes. Ann Arbor Science. Ann Arbor, MI. 441 p.

20. APHA, AWWA, WPCF. 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed.. APHA, AWWA, WPCF. Washington, D.C. 1268 p.

21. Goerlitz, D. F., L. M. Law. 1974. Determination of Chlorinated Insecticides in Suspended Sediment and Bottom Material. Journal of the AOAC. 57:176-181.

22. Brown, J. F., R. E. Wagner, J. C. Carnahan. 1987. Environmental Transformations of PCBs. General Electric Company Research and Development for the Destruction of PCBs, Sixth Progress Report. Chpt 6:35-41.

23. Higuchi, K. 1976. PCB Poisoning and Pollution. Tokyo: Kodansha; N.Y.: Academic Press. 184 p.

APPENDICES

REACTOR LIQUID COD DATA

Running Time (days)	Absorbance	Diluted COD (mg/L)	Actual COD (mg/L)	Average COD (mg/L)	Percent COD Removal	Feed COD (mg/L)
-14.76	0.220	300.52	6010.3	6023.9	2.45	6175
	0.221	301.87	6037.5			
-14.02	0.210	286.94	5738.9	5738.9	7.06	6175
	0.210	286.94	5738.9			
-12.97	0.188	257.08	5141.7	5168.8	16.29	6175
	0.190	259.80	5196.0			
-12.00	0.172	235.37	4707.4	4666.7	24.43	6175
	0.169	231.30	4626.0			
-10.76	0.158	216.37	4327.4	4354.5	29.48	6175
	0.160	219.08	4381.7			
-10.00	0.153	209.58	4191.6	4191.6	32.12	6175
	***	ERR	ERR			
-9.01	0.140	191.94	3838.8	3974.5	35.64	6175
	0.150	205.51	4110.2			
-8.01	0.124	170.22	3404.5	3485.9	43.55	6175
	0.130	178.37	3567.3			
-7.01	0.152	208.22	4164.5	4259.5	31.02	6175
	0.159	217.73	4354.5			
-6.02	0.148	202.80	4055.9	3838.8	37.83	6175
	0.132	181.08	3621.6			
-5.01	0.156	213.65	4273.1	4218.8	29.35	5971
	0.152	208.22	4164.5			
-4.05	0.124	170.22	3404.5	3377.3	43.44	5971
	0.122	167.51	3350.2			
-3.01	0.118	162.08	3241.6	3268.7	45.26	5971
	0.120	164.79	3295.9			
-2.05	0.112	153.94	3078.7	3078.7	48.44	5971
	0.112	153.94	3078.7			
-1.01	0.111	152.58	3051.6	3051.6	48.89	5971
	0.111	152.58	3051.6			
0.00	0.111	152.58	3051.6	3038.0	49.12	5971
	0.110	151.22	3024.4			
0.99	0.120	164.79	3295.9	3309.4	44.57	5971
	0.121	166.15	3323.0			
1.99	0.119	163.44	3268.7	3282.3	45.03	5971
	0.120	164.79	3295.9			
2.94	0.120	164.79	3295.9	3268.7	45.26	5971
	0.118	162.08	3241.6			
3.96	0.116	159.36	3187.3	3119.4	47.76	5971
	0.111	152.58	3051.6			
5.00	0.116	159.36	3187.3	3160.2	53.15	6745
	0.114	156.65	3133.0			
5.95	0.109	149.86	2997.3	2943.0	56.37	6745
	0.105	144.44	2888.7			
7.98	0.099	136.29	2725.8	2739.4	59.39	6745
	0.100	137.65	2753.0			
8.97	0.210	286.94	3156.4	3156.4	53.20	6745
	0.210	286.94	3156.4			
11.02	0.199	272.01	2992.2	2925.0	56.63	6745

REACTOR LIQUID COD DATA (continued)

Running Time (days)	Absorbance	Diluted COD (mg/L)	Actual COD (mg/L)	Average COD (mg/L)	Percent COD Removal	Feed COD (mg/L)
	0.190	259.80	2857.8			
11.98	0.188	257.08	2827.9	2805.5	58.10	6695
	0.185	253.01	2783.1			
14.97	0.195	266.59	2932.4	2969.8	55.64	6695
	0.200	273.37	3007.1			
17.96	0.200	273.37	3007.1	3022.0	54.86	6695
	0.202	276.09	3036.9			
19.91	0.202	276.09	3036.9	3051.9	54.42	6695
	0.204	278.80	3066.8			
21.93	0.199	272.01	2992.2	3059.3	54.30	6695
	0.208	284.23	3126.5			
22.93	0.218	297.80	3275.8	3208.6	52.07	6695
	0.209	285.59	3141.4			
25.02	0.206	281.51	3096.7	3134.0	53.19	6695
	0.211	288.30	3171.3			
26.93	0.215	293.73	3231.0	3193.7	56.38	7322
	0.210	286.94	3156.4			
32.02	0.220	300.52	3305.7	3365.4	54.04	7322
	0.228	311.37	3425.1			
33.93	0.220	300.52	3305.7	3305.7	30.57	4761
	0.220	300.52	3305.7			
35.96	0.190	259.80	2857.8	2857.8	39.97	4761
	0.190	259.80	2857.8			
38.00	0.161	220.44	2424.8	2432.3	48.92	4761
	0.162	221.80	2439.8			
40.99	0.142	194.65	2141.2	2193.4	53.93	4761
	0.149	204.15	2245.7			
43.00	0.115	158.01	1738.1	1760.5	63.02	4761
	0.118	162.08	1782.9			
46.01	0.080	110.50	1215.6	1290.2	72.90	4761
	0.090	124.08	1364.8			
48.96	0.070	96.93	1066.3	1058.8	77.76	4761
	0.069	95.58	1051.3			
52.93	0.040	56.22	618.4	618.4	87.01	4761
	0.040	56.22	618.4			
55.97	0.050	69.79	767.7	700.5	86.57	5217
	0.041	57.57	633.3			
59.92	0.039	54.86	603.4	610.9	88.29	5217
	0.040	56.22	618.4			
62.93	0.041	57.57	633.3	633.3	87.86	5217
	b.d.	ERR	ERR			
67.93	0.012	18.21	200.4	215.3	92.47	2858
	0.014	20.93	230.2			
73.85	0.014	20.93	230.2	207.8	92.73	2858
	0.011	16.86	185.4			
76.96	0.022	31.79	190.7	182.6	94.10	3092
	0.020	29.07	174.4			3092
80.97	0.050	69.79	418.7	422.8	86.33	3092
	0.051	71.15	426.9			3092

REACTOR LIQUID COD DATA (continued)

Running Time (days)	Absorbance	Diluted COD (mg/L)	Actual COD (mg/L)	Average COD (mg/L)	Percent COD Removal	Feed COD (mg/L)
83.90	0.045	63.00	378.0	361.7	88.30	3092
	0.041	57.57	345.4			3092
87.95	0.050	69.79	348.9	348.9	88.72	3092
	0.050	69.79	348.9			3092
90.98	0.042	58.93	294.7	287.9	90.69	3092
	0.040	56.22	281.1			3092
95.97	0.120	164.79	824.0	851.1	72.48	3092
	0.128	175.65	878.3			3092
102.17	0.029	41.29	206.4	179.3	94.20	3092
	0.021	30.43	152.1			
122.29	0.152	208.22	1041.1	1034.3	67.84	3216
	0.150	205.51	1027.6			
124.99	0.151	206.87	1034.3	1030.9	67.94	3216
	0.150	205.51	1027.6			
128.31	0.159	217.73	1088.6	1088.6	66.22	3223
	evap	ERR	ERR			
130.35	0.203	277.44	1387.2	1353.3	58.01	3223
	0.193	263.87	1319.4			
131.96	0.219	299.16	1495.8	1441.5	55.27	3223
	0.203	277.44	1387.2			
134.22	0.235	320.87	1604.4	1587.4	53.85	3440
	0.230	314.09	1570.4			
136.99	0.209	285.59	1427.9	1397.4	59.38	3440
	0.200	273.37	1366.9			
140.00	0.245	334.45	1672.2	1689.2	50.90	3440
	0.250	341.23	1706.2			
144.08	0.240	327.66	1638.3	1621.3	49.96	3240
	0.235	320.87	1604.4			
147.05	0.226	308.66	1543.3	1529.7	52.78	3240
	0.222	303.23	1516.2			
151.95	0.249	339.88	1699.4	1699.4	47.55	3240
	evap	ERR	ERR			
155.99	0.248	337.84	1689.2	1689.2	47.86	3240
160.96	0.199	272.01	1360.1	1360.1	58.02	3240
166.89	0.214	292.37	1461.9	1461.9	55.58	3291
171.43	0.242	330.37	1651.9	1651.9	49.80	3291
175.88	*	ERR	ERR	ERR	ERR	3291
179.99	0.228	311.37	1556.9	1556.9	52.69	3291
185.90	0.290	395.52	1977.6	1977.6	39.90	3291
190.13	0.354	482.38	2411.9	2411.9	26.71	3291
194.88	0.305	415.88	2079.4	2079.4	36.81	3291
200.92	0.317	432.17	2160.8	2160.8	33.58	3253
206.19	0.294	400.95	2004.8	2004.8	38.38	3253
209.84	0.338	460.67	2303.3	2303.3	29.20	3253
214.96	0.305	415.88	2079.4	2079.4	36.09	3253
219.96	0.302	411.81	2059.0	2059.0	36.71	3253

Filename: COD

REACTOR LIQUID PCB CONCENTRATION DATA

Sample Time (days)	Tetra	Penta	Hexa	Hepta	Octa
	*********	Concentration	(ug/l)	*************	
122.75	7.3137	7.2013	5.3045	4.4894	1.8516
125.45	6.5724	6.7993	5.0167	4.3322	1.7854
130.81	4.7962	4.6911	3.7229	3.6584	1.3326
132.43	3.7600	3.3694	3.1550	3.4360	1.0575
137.45	3.9406	4.0147	3.3090	3.5301	1.2039
140.46	3.5052	3.5487	3.0787	3.2922	1.2892
144.54	3.4564	3.2551	2.8278	2.8571	1.0990
147.51	2.9990	3.0892	2.7784	2.9021	1.0514
152.42	2.6658	2.7148	2.4030	3.0195	0.8227
156.45	2.2990	1.8786	2.3110	3.0324	0.7738
161.42	3.2646	2.5724	2.0423	2.8564	0.5039
167.35	5.6590	5.7518	5.0465	5.0544	3.0330
171.88	3.7978	3.7998	3.5729	3.7697	1.7465
176.34	3.6157	3.8237	3.4545	3.6742	1.6590
180.45	3.0238	2.8932	2.5743	2.8691	0.8598
186.36	3.1865	3.0943	3.0056	3:1338	1.1665
190.59	3.3323	3.1995	3.0645	3.1904	1.3640
195.34	2.7340	2.6408	2.6415	2.9252	1.0259
197.36	3.4740	3.1048	2.6240	2.7608	0.8192
201.38	3.1980	4.0421	4.0968	4.2704	1.1342
206.65	2.9004	3.0846	3.6545	5.4985	1.0858
209.48	2.9262	3.5441	3.6021	4.5918	2.0287
209.74	2.6709	3.2964	3.4958	4.6008	0.9602
209.97	2.5875	3.0832	3.7947	4.9919	1.1263
210.30	3.1539	3.7980	4.0838	5.1900	1.1148
210.65	3.4750	4.3767	4.9397	5.4624	1.1245
210.97	3.2935	4.0234	4.7097	5.8379	1.2545
211.32	2.8613	3.8415	3.8707	4.9911	1.0981
211.65	2.9289	3.7200	3.9461	5.1285	1.1407
211.98	2.8519	3.6604	3.7040	4.9091	0.9562
212.46	2.7751	2.6651	3.9061	5.1818	1.1486
212.85	3.0256	3.7166	4.0148	5.1552	1.0348
213.39	2.8887	3.4983	3.7208	4.9335	1.0621
213.75	3.0891	3.7553	3.6241	5.0130	0.9410
214.36	2.9269	3.4275	3.6471	4.6986	0.9367
214.84	2.7165	3.2886	3.3566	4.5764	0.9351
215.42	3.5303	3.9471	4.7864	5.6852	2.0361
216.49	2.8648	3.6288	3.9986	5.1121	1.1195
217.35	3.1858	3.8641	4.2921	5.3110	1.2652
218.35	3.4537	4.2169	4.4914	5.2379	1.1644
220.42	3.0878	3.6685	3.5856	4.5200	1.0049

Filename: PCB-CONC

REACTOR LIQUID PCB PERCENT REMOVAL DATA

Sample Time (days)	Tetra	Penta	Hexa	Hepta	Octa
	*********** Per Cent Removal ***************				
62.90	91.98	91.77	94.05	94.94	95.15
67.93	94.84	94.55	95.68	96.21	96.35
73.84	93.60	93.28	94.63	95.23	95.44
76.96	92.89	92.73	94.28	94.89	95.71
80.97	93.14	92.87	94.65	94.98	95.95
83.90	91.82	91.09	93.06	93.51	94.36
90.98	87.98	85.95	89.65	89.93	90.29
95.97	91.89	90.55	92.41	92.79	93.12
122.75	93.35	93.45	95.18	96.26	98.32
125.45	68.70	67.62	76.11	80.31	91.50
130.81	77.16	77.66	82.27	83.37	93.65
132.43	82.10	83.96	84.98	84.38	94.96
137.45	81.24	80.88	84.24	83.95	94.27
140.46	83.31	83.10	85.34	85.04	93.86
144.54	83.54	84.50	86.53	87.01	94.77
147.51	85.72	85.29	86.77	86.81	94.99
152.42	87.31	87.07	88.56	86.27	96.08
156.45	89.05	91.05	89.00	86.22	96.32
161.42	84.45	87.75	90.27	87.02	97.60
167.35	73.05	72.61	75.97	77.03	85.56
171.88	81.92	81.91	82.99	82.87	91.67
176.34	82.78	81.79	83.55	83.30	92.10
180.45	85.60	86.22	87.74	86.96	95.91
186.36	84.83	85.27	85.69	85.76	94.45
190.59	84.13	84.76	85.41	85.50	93.50
195.34	86.98	87.42	87.42	86.70	95.11
197.36	83.46	85.22	87.50	87.45	96.10
201.38	84.77	80.75	80.49	80.59	94.60
206.65	86.19	85.31	82.60	75.01	94.83
209.48	97.34	96.78	96.73	96.17	98.16
209.74	97.57	97.00	96.82	96.17	99.13
209.97	97.65	97.20	96.55	95.84	98.98
210.30	97.13	96.55	96.29	95.67	98.99
210.65	96.84	96.02	95.51	95.45	98.98
210.97	97.01	96.34	95.72	95.14	98.86
211.32	97.40	96.51	96.48	95.84	99.00
211.65	97.34	96.62	96.41	95.73	98.96
211.98	97.41	96.67	96.63	95.91	99.13
212.46	97.48	97.58	96.45	95.68	98.96
212.85	97.25	96.62	96.35	95.70	99.06
213.39	97.37	96.82	96.62	95.89	99.03
213.75	97.19	96.59	96.71	95.82	99.14
214.36	97.34	96.88	96.68	96.08	99.15
214.84	97.53	97.01	96.95	96.19	99.15
215.42	96.79	96.41	95.65	95.26	98.15
216.49	97.40	96.70	96.36	95.74	98.98
217.35	97.10	96.49	96.10	95.57	98.85
218.35	96.86	96.17	95.92	95.64	98.94
220.42	97.19	96.66	96.74	96.23	99.09

Filename: PCB-CONC

REACTOR LIQUID pH DATA

Date	Running Time (days)	pH
----	------	---
09-Aug	3	7.51
13-Aug	7	7.90
15-Aug	9	8.01
20-Aug	14	7.50
21-Aug	15	7.54
23-Aug	17	7.35
26-Aug	20	7.22
27-Aug	21	6.95
28-Aug	22	6.88
29-Aug	23	6.75
30-Aug	24	6.66
31-Aug	25	6.45
01-Sep	26	6.45
02-Sep	27	6.45
05-Sep	30	6.20
07-Sep	32	6.10
08-Sep	33	6.05
09-Sep	34	6.07
10-Sep	35	6.11
11-Sep	36	6.17
12-Sep	37	6.13
13-Sep	38	6.16
14-Sep	39	6.18
15-Sep	40	6.24
16-Sep	41	6.33
17-Sep	42	6.35
18-Sep	43	6.48
19-Sep	44	6.47
20-Sep	45	6.54
21-Sep	46	6.63
22-Sep	47	6.48
23-Sep	48	6.61
24-Sep	49	6.70
25-Sep	50	6.68
28-Sep	53	6.73
29-Sep	54	6.72
01-Oct	56	6.75
02-Oct	57	6.73
03-Oct	58	6.73
04-Oct	59	6.74
05-Oct	60	6.79
06-Oct	61	6.75
07-Oct	62	6.75
08-Oct	63	6.74
09-Oct	64	6.77
11-Oct	66	6.94
12-Oct	67	6.94
13-Oct	68	6.96
14-Oct	69	6.91

Date	Running Time (days)	pH
----	------	---
15-Oct	70	6.89
16-Oct	71	6.90
17-Oct	72	6.88
18-Oct	73	6.86
19-Oct	74	6.87
20-Oct	75	6.89
21-Oct	76	6.88
22-Oct	77	6.86
23-Oct	78	6.86
25-Oct	80	6.82
26-Oct	81	6.83
27-Oct	82	6.83
29-Oct	84	6.82
30-Oct	85	6.82
31-Oct	86	6.80
01-Nov	87	6.80
02-Nov	88	6.79
03-Nov	89	6.78
04-Nov	90	6.78
06-Nov	92	6.84
09-Nov	95	6.79
11-Nov	97	6.79
12-Nov	98	6.79
13-Nov	99	6.80
14-Nov	100	6.82
15-Nov	101	6.80
16-Nov	102	6.88
17-Nov	103	6.79
18-Nov	104	6.73
20-Nov	106	6.78
22-Nov	108	6.75
04-Dec	120	6.74
06-Dec	122	6.74
14-Dec	130	6.78
16-Dec	132	6.76
18-Dec	134	6.84
21-Dec	137	6.80
24-Dec	140	6.80
28-Dec	144	6.75
31-Dec	147	6.78
14-Jan	161	6.81
24-Jan	171	6.72
29-Jan	176	6.72
01-Feb	179	6.82
08-Feb	186	[illegible]
12-Feb	190	6.89
17-Feb	195	6.87
19-Feb	197	6.66
03-Mar	210	6.80

Date	Running Time (days)	pH
----	------	---
04-Mar	211	[illegible]
08-Mar	215	[illegible]

Filename: pH