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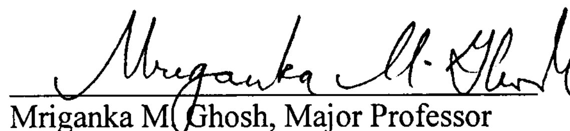

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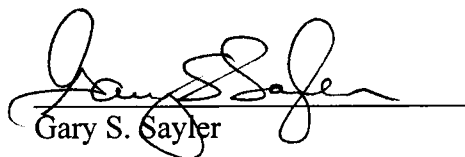

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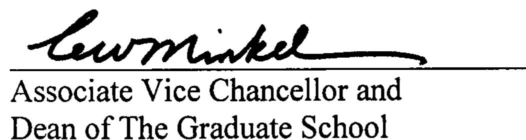

Associate Vice Chancellor and
Dean of The Graduate School

AEROBIC BIODEGRADATION OF PCBS IN PHOTOLYZED AND NON-PHOTOLYZED SURFACTANT SOLUTIONS

A Thesis Presented for the Master of Science Degree

The University of Tennessee, Knoxville

Keith A. La Torre
May, 1998

Dedication

This thesis is dedicated to my mother,

Carol La Torre

my father,

Andrew La Torre

and my sister,

Kim Ensley.

Thanks for your love, patience and teaching

Acknowledgements

I would like to thank my advisor, Dr. Mriganka Ghosh, for selecting me as a graduate research assistant. I would also like to thank him for his mentoring, patience, and insight. Also, I am grateful to Dr. Gary S. Sayler for inviting me into for creating The Center for Environmental Biotechnology, a wonderful place to work and to research. In addition to I would like to thank Dr. Chris Cox for accepting my request to be apart of my committee.

Other people who were instrumental in my research were Dr.'s Alice Layton and Curtis Lajoie. Both tirelessly answered questions, listened to my ideas, and focused my thoughts. Also, Zhou Shi always made time, at his expense, to answer my questions with extreme thoroughness. I am a better scientist due to the help I received from all of the peopled mentioned.

Finally, I like to thank all persons at The Center for Environmental Biotechnology. All were extremely helpful, friendly, and insightful.

Abstract

The enhancement of aerobic biodegradation of polychlorinated biphenyls (PCBs) by UV irradiation was investigated in batch reactors. The PCBs were dissolved in a micellar solution of a nonionic surfactant, polyoxyethylene 10 lauryl ether [POL (10)]. UV irradiation was used to reductively dechlorinate highly chlorinated PCB congeners to make them more vulnerable to aerobic biochemical attack. In an integrated bioremediation scheme, (1) surfactants solubilize PCBs; (2) photolysis is used to make highly chlorinated congeners more amenable to biodegradation; and finally, (3) genetically engineered aerobic microorganisms (GEMs), capable of using surfactants for growth while cometabolizing PCBs, are used for biodegradation. At a POL (10) dosage of 10 g/L, percent degradation ranged from 52-64% in PCB solutions containing 22-269 mg/L of total PCB, the maximum removal occurring in a solution containing 46 mg/L of PCB. However, the rate of biodegradation was best for the 269 mg/L sample (18.4 mg/L-day). For 50 mg/L PCB solutions in 0.5 - 10 g/L POL (10), a maximum biodegradation of 74% was obtained at a POL (10) concentration of 2 g/L. The 1 g/L sample gave the highest rate of biodegradation of 15.4 mg/L-day. With UV irradiation for 40 min, 63% of the PCBs in a 210 mg/L solution could be degraded, and an additional degradation of 60% of the remainder was realized in a subsequent biodegradation step. By comparison, only 52% of PCBs could be aerobically biodegraded without photolysis.

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List of Abbreviations

POL (10) – polyoxyethylene 10 lauryl ether

PCBs – polychlorinated biphenyls

GEMs – genetically engineered microorganisms

CPRG – chlorophenol red –β-D-galactopyranoside

K_{ow} – octanol/water partitioning coefficient

CMC – critical micelle concentration

bphA – biphenyl 2,3 dioxygenase

bphB – dihydrodiol dehydrogenase

bphC – 2,3 dihydroxybiphenyl dioxygenase

bphD – 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid

2,4 D – 2,4-dichlorophenoxyacetic acid

FAVs – field application vectors

KC500 – Kaneclor 500

DI – de-ionized

UV – ultraviolet

DO – dissolved oxygen

ECD – electron capture detector

GC – gas chromatography

CTAS – cobaltothiocyanate active substance

MSR – molar solubility ratio

K_m – micellar partitioning coefficient

q – specific substrate utilization rate

CBA – chlorobenzoate

K_{do} – half-saturation constant for dissolved oxygen

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are synthetic organic chemicals that consist of a biphenyl backbone and between 1 and 10 chlorine atoms substituting for hydrogen atoms on the biphenyl ring (**Figure 1**). Depending on the number and position of the chlorine atoms, 209 different PCB molecules are possible, each referred to as a congener (Bedard and Quensen, 1995).

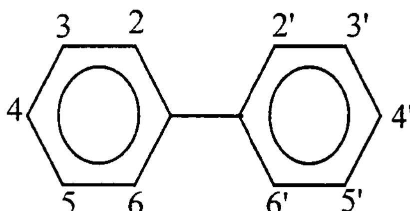


Figure 1. PCB molecule with numbers representing positions of chlorine atoms

PCBs were synthesized by catalytic chlorination of biphenyl creating mixtures of PCB congeners distinguished by their molecular weights (Bedard and Quensen, 1995). Companies such as Monsanto manufactured PCBs under the trade name 'Aroclor' with varying weight percents of chlorine. For example, Aroclor 1242 contains 12 carbon atoms and 42% chlorine by weight. Likewise Aroclor 1254 has 12 carbons and has 54% chlorine by weight. Between 1929 and 1978, approximately 1.4 billion pounds of PCBs were manufactured. PCBs have many important chemical properties, such as chemical and thermal stability, non-flammability, and high electrical resistance among them (Unterman, 1996; Bedard and Quensen, 1995). PCBs are ideal chemicals for use as

dielectric fluids in capacitors and transformers, lubricants, coolants, solvent extenders, and as flame retardants (Unterman, 1996; Bedard and Quensen, 1995; Sylvestre and Sondossi, 1994).

PCBs are of environmental concern today because of their ubiquity in the environment, their hydrophobicity, their recalcitrance to natural attenuation, and their possible adverse effects to humans and ecology. It is estimated that several million pounds of PCBs have entered the environment (Bedard and Quensen, 1995). The hydrophobicity of PCBs makes them resistant to normal ecological flushing characteristic of more water soluble compounds. Due to bioaccumulation, PCB levels in fish and human breast milk have reached toxic levels in some instances (Safe, 1989). PCBs partition mainly into the fatty tissues of animals. The higher up the food chain, the more susceptible the organism is to these toxic levels of contaminants (Hooper *et* al., 1990). Even in natural waters with low aqueous concentrations of PCBs extremely high levels of PCBs can be found in the fatty tissues of fish (Hooper *et* al., 1990). The combined effects of slow degradation, bioaccumulation, and toxicity make PCBs a persistent problem.

In the natural environment and in laboratory studies, anaerobic microbial processes have been shown to biodegrade PCBs by reductive dechlorination (Brown *et al.*, 1987, Morris *et al.* 1993, Commandeur and Parsons, 1990, Williams, 1994), but this process is slow. Aerobic biodegradation of PCBs occurs more rapidly, but aerobic microbial processes usually cannot degrade highly chlorinated PCB congeners with more than four substituted chlorine atoms (Parson *et al.*, 1988; Adriens *et al.*, 1989; Brunner *et al.*, 1985;

Masse *et al.*, 1984; Shields *et al.*, 1985; Hickey *et al.*, 1993; Commandeur and Parsons, 1990), especially if they are located at the *ortho* positions (2,2',6, and 6') (Furukawa *et al.* 1978, Bedard and Haberl, 1990). Reportedly, there has been some success in the biodegradation of penta- and hexachlorbiphenyls (Bopp, 1986; Bedard *et al.*, 1986; Furukawa 1982, Bedard and Haberl, 1990, Commandeur *et al.*, 1995). Most research on aerobic biodegradation of PCBs has been done in aqueous solutions as opposed to surfactant or solvent solutions. Due to the low solubility of PCBs (Sawhney, 1986), only approximately 200 μg/L of PCBs may be available for possible biodegradation by the aerobic organisms (Huntzinger *et* al., 1974). The biodegradation of PCBs in surfactant solutions has been shown to increase due to increased solubility (Lajoie *et al.*, 1993, Lajoie *et al.*, 1994, Lajoie *et al.*, 1997).

There are a number of options to remediate PCB contaminated soils. These soils can be excavated and landfilled in an appropriate hazardous waste storage facility; the soils can be incinerated; or the soil-bound PCBs can be extracted and this extract can be incinerated or landfilled. All of these options tend to be quite expensive, although incineration has been shown to be very effective, removing 99.9999% of the PCBs. Other processes are currently being researched in an attempt to make remediation of PCB-contaminated soils less expensive, particularly those that do not include excavation of soil. To remediate a contaminated site efficiently, the PCBs would first have to be extracted from the soil. Surfactants have been shown to greatly increase the solubility of hydrophobic environmental contaminants (Edwards *et al.*, 1994; Yeom *et al.*, 1995; Yeom *et al.*, 1996; Sun *et al.*, 1995). Surfactants have also been shown to extract PCBs

from soils, particularly when the surfactant concentration is above the critical micellar concentration (CMC) (Abdul *et al.*, 1992). This extraction can either be done ex situ with excavated soils, or in situ by flushing the soils in place with the surfactant. The ensuing surfactant/PCB solution would then have to be treated. It is possible that a sequential photolysis/biodegradation system can be used.

Photolysis with UV light has been shown to degrade and/or dechlorinate PCBs. It works particularly well with the biorecalcitrant, *ortho*-substituted congeners as well as the highly chlorinated congeners in aqueous solutions (Sawhney, 1986; Safe, *et al.*, 1975; Dullin *et al.*, 1986;) and in surfactant solutions (Chu and Jafvert, 1994; Epling *et al.*, 1988; Shi *et al.*, 1994). The use of both UV irradiation and biodegradation has been shown to degrade PCBs (Kong and Sayler, 1983; Shimura *et* al., 1996; Baxter and Sullivan 1984). Aerobic, genetically engineered microorganisms (GEMs) can be used to degrade surfactants and simultaneously co-metabolize PCB congeners remaining in solution (Lajoie *et al.*, 1993, 1994, 1997; Layton *et al.*, 1994a, 1994b). The goal of this research was to optimize an integrated scheme involving surfactant-washing, photolysis, and aerobic biodegradation for remediating PCB-contaminated soils.

2. OBJECTIVES

Remediation of PCBs can be expensive, inefficient, and ineffective. This research was conducted in an attempt to improve the remediation of PCBs. The specific objectives of the study were:

- To study the solubilization of Aroclor 1242 by nonioinic surfactants
- To study the aerobic biodegradation of PCBs in surfactant solutions
- To study the aerobic biodegradation of photolyzed PCBs in surfactant solutions
- To study the toxicology of PCB biodegraded solutions

3. SCOPE OF THE STUDY

All experiments in this study were conducted using a single non-ionic surfactant, polyoxyethylene 10 lauryl ether (POL (10)) and a commercial PCB mixture, Aroclor 1242. A mixture of two PCB-GEMs, *Pseudomonas putida* IPL5::TnPCB and *Ralstonia eutropha* B30P4::TnPCB, were used in all biodegradation studies. Control bioreactors were run in parallel using a mixture of the GEMs, *Psuedomonas putida* IPL5::TnTc and *Ralstonia eutropha* B30P4::TnTc. The control strains were not capable of producing PCB degrading enzymes. The surfactant plays a dual role in this treatment scheme. It enhances the solubility of PCBs and hence their bioavailability, it also provides the sole carbon source for growth for the GEMs which fortuitously co-metabolize PCBs. Biodegradation of PCBs can be limited by the amount and position of the substituted chlorines. UV-irradiation can be used to reductively dechloriante PCBs and make them more amenable to biological attack. Also, the use of toxicological assays were used to determine if biodegradation decreased overall toxicity. This study compared PCB removal from an Aroclor 1242 solution in pure POL (10) by means of three techniques: 1) biodegradation, 2) photolysis, 3) sequential photolysis/biodegradation.

4. BACKGROUND

4.1 PCBs: The Recent History

The environmental impact of PCBs became an important issue starting in the 1960s. Jensen (1966) first reported the significance of toxicity of these chemicals to wildlife. In 1968, the acute toxicity of PCBs to humans became manifest when, in Japan, 138 people ingested rice oil contaminated with PCBs. It caused illnesses such as chloracne, discoloration of the gums, joint swelling, waxy secretions from glands in the eyelids, and melanin disturbances affected many others; twenty-two eventually died within five years (Cairns *et al.*, 1986; Hooper *et al.*, 1990). Another accidental contamination of PCBs in cooking oil showed abnormalities in the people's skin, teeth, lungs, and nails, and the development of infants born to exposed mothers was delayed relative to unexposed mothers (Hooper *et al.*, 1990). By 1976, the US Congress enacted the Toxic Substances Control Act (TSCA). The EPA was directed to control the manufacture, processing, distribution, use, disposal and labeling of PCBs (McCoy, 1989), among other toxic substances. By July of 1979, the manufacture of PCBs were totally banned in the US under TSCA (Hooper *et al.*, 1990).

The basis for the regulation of PCBs is its apparent toxicity towards humans and wildlife. PCBs evoke a variety of toxic responses in both humans and laboratory animals (Mousa *et al.*, 1996, Safe *et al.*, 1994, Schmelling, 1998). The toxic effects of PCBs on wildlife are documented in numerous studies. Eagle populations in Europe and the United States

declined in the recent past due to the reduced breeding rate induced by the high levels of PCBs (Hernadez *et al.*, 1989). Many other deformities and toxic effects have been documented in birds, reptiles, mammals, and fish as a result of extended PCB exposure and bioaccumulation (Muccini, 1997). Furthermore, even plants uptake PCBs. This leads not to malformation of the plant, rather this uptake leads to access of PCBs to the food chain (Sawhney and Hankin, 1984; Webber *et al.*, 1994; O'Conner *et al.*, 1990).

However, the most important toxic effect of PCBs on humans is chronic rather than acute. In laboratory experiments with primates and rats, PCBs reduced the cellular levels of dopamine, affecting neurotransmitters in the brain (Tilson and Kodavanti, 1997; Angus *et al.*, 1997). Such neurological effects are of particular concern for the developing human/animal (Tilson and Kodavanti, 1997; Eriksson, 1997). An epidemiological study compared the neurological effects of prenatal exposure of PCBs from cohorts in Michigan and North Carolina. The children in Michigan, more exposed to PCBs than those in North Carolina, were found to be twice as likely to perform poorly on IQ tests(Jacobson and Jacobson, 1997). Another epidemiological study, based on 242 male workers employed by the capacitor manufacturing industry during 1965 and 1978 (Gustavsson and Hogstedt, 1997), found a significant increase in the risk of cancer of the liver and bile ducts in the subjects.

4.1.1 Structure and Toxicity

The toxicity of the PCBs depends on the mixtures and/or congeners present. Specific PCB congeners vary widely in its toxicity. Congeners with no *ortho* or mono-*ortho* substitutions which are strucuturally similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin are directly toxic (Sylvestre and Sondossi, 1994). These compounds tend to be planar in configuration. Other congeners that are partially co-planar and contain between 5 to 7 chlorine atoms, mostly in the *para* or *meta* position, also tend to be toxic (Sylvestre and Sondossi, 1994). Only 15 to 20 congeners are documented to be significantly toxic (**Table 1**) (Sylvestre and Sondossi, 1994).

Table 1. List of the most toxic PCB congeners[1] (Syvestre and Sondossi, 1994).

Group 1a[2]		Group 1b[3]		Group 2[4]	
IUPAC No.	Structure	IUPAC No.	Structure	IUPAC No.	Structure
77	3,3',4,4'	105	2,3,3',4,4'	87	2,2',3,4,5'
126	3,3',4,4',5	118	2,3',4,4',5	99	2,2',4,4',5
169	3,3',4,4',5,5'	128	2,2',3,3',4,4'	101	2,2',4,5,5'
		138	2,2',3,4,5',5'	153	2,2',4,4'5,5'
		156	2,3,3',4,4',5'	180	2,2',3,4,4',5,5'
		170	2,2',3,3',4,4',5	183	2,2',3,4,4',5,6
				194	2,2',3,3',4,4',5,5'

[1]The PCB congeners are classified following McFarland and Clarke priority group
[2]Group 1a includes the three most potent congeners (pure 3-methylcholanthrene-type inducers)
[3]Group 1b comprises mixed type inducers that have been reported frequently in environmental samples
[4]Group 2 comprises phenobarbitol-type inducers that have high abundance in avian and mammalian samples

4.1.1.1 Assay of Estrogenic Activity

Recently, research has focused on the impact of chemicals in the environment that mimic the female hormone estrogen. Hence, these chemicals are termed "environmental estrogens." Aroclor 1221 (a lightly chlorinated mixture of PCBs) exhibits estrogenic activity in female rats (Gellert, 1978). Exposure to environmental estrogens may increase the risk of some cancers by disrupting the normal endocrine function (Gaido *et al.*, 1997). Of particular concern is the connection between the environmental estrogens, such as PCBs and DDT, and breast cancer (Safe, 1995). However, a recently published study of 240 women with breast cancer and 236 women without breast cancer refuted the connection between PCBs and breast cancer. The median level of PCBs in the control (non-breast cancer) women were actually higher than in the experimental group (women with breast cancer) (Hunter *et al.*, 1997).

To quickly evaluate the estrogenicity of a compound, a rapid and easily performed assay was needed. An assay was developed to determine the interactions of the contaminant with estrogen, androgen, and progesterone receptors using yeast (Gaido *et al.*, 1997). Some chemicals have the ability to interact directly with steroid receptors either mimicking or blocking steroid action (Gaido *et al.*, 1997). Mammalian steroid receptors were introduced into the yeast strain *Saccharomyces cerevisae* (Gaido *et al.*, 1997). Upon binding, the estrogen-occupied receptor interacts with transcription factors causing expression of the reporter gene *lac-Z* (Routledge and Sumpter, 1996). The enzyme produced, B-galactosidase, reacts with chlorophenol red-B-D-galactopyranoside (CPRG).

CPRG is normally yellow, but upon reaction with, B-galactosidase, a color results which can be colorimetrically measured (Routledge and Sumpter, 1996).

4.1.2 Chemistry

PCBs are chlorinated derivatives of biphenyl, which are sparingly soluble in water but highly soluble in nonpolar solvents (Gamble, 1986). The PCB mixtures used in industry, such as Aroclor 1242, are colorless viscous fluids with low solubility and can withstand high temperature without degradation (Hutzinger *et al.*, 1974). The general physical and toxicological properties of Aroclor mixtures are listed in **Table 2**. These mixtures are made by a combination of congeners, and there are 209 possible congener combinations varying in the number and position of the chlorine substitutions (**Table 3**). The solubility of PCBs in water generally decreases with increases in the degree of chlorination (Sawhney, 1986).

Table 2. General physical properties of various Aroclors (Cairns *et al.*, 1986)

Aroclor No.	Form	Sp. Gravity	Distillation Range (°C)
1221	Clear, mobile oil	1.182-1.192	275-320
1232	Clear, mobile oil	1.270-1.280	290-325
1242	Clear, mobile oil	1.381-1.392	325-366
1248	Clear, mobile oil	1.405-1.415	340-375
1254	Light yellow viscous oil	1.495-1.505	365-390
1260	Light yellow, soft sticky resin	1.555-1.566	385-420
1262	Light yellow sticky viscous resin	1.572-1.583	395-425
1268	White to off-white powder	1.804-1.811	435-450

Table 3. Number of possible congeners of PCBs (Cairns *et al.*, 1986)

Chlorine Substitution	Number of Possible Isomers
Mono-	3
Di-	12
Tri-	24
Tetra-	42
Penta-	46
Hexa-	42
Hepta-	24
Octa-	12
Nona-	3
Deca-	1
Total	209

4.1.3 Environmental Fate and Transport

Nonpolarity, low aqueous solubility, high stability, and recalcitrance to physical and biological degradation are some of the qualities that made PCB a wonderful industrial chemical. These same characteristics contributed to the persistence and accumulation of PCBs in the environment.

The hydrophobicity of PCBs is an important factor determining the fate of PCBs in the environment. A number of studies have shown that sorption of PCBs by sediments and soils is related to their total organic carbon (Sawhney, 1986). Therefore, the partitioning of PCBs, between water and an immiscible solvent, should correspond to the partitioning of PCBs between soil and water (Sawhney, 1986). The octanol/water partitioning coefficient (K_{ow}) measures the partitioning of a compound between the water and octanol phases, and it is commonly used as a measure of hydrophobicity (Hooper *et al.*, 1990).

The K_{ow} is also used as an estimate of the amount of partitioning between sediments and water (Hooper *et al.*, 1990). The K_{ow} for PCBs range six orders of magnitude, 10 to 10^7 (Sawhney, 1986). The average log K_{ow} for monochloro-PCBs is 4.5, whereas that for congeners with seven chlorine substitutions was reported to be 8.1 (Rapaport and Eisenreich, 1984). Chemicals with high K_{ow} values have a predilection for surface boundaries, such as the air-water and soil-water interfaces, as well as lipids (Hooper *et al.*, 1990). Their hydrophobic, nonpolar nature causes PCBs to accumulate on soil surfaces. Unless actively remediated, contaminated soils may serve as permanent repositories for future water contamination.

4.2 Surfactants in Soil Remediation

Surfactants are amphiphilic chemicals which contain both hydrophobic and hydrophilic moieties. Surfactants increase the solubility of hydrophobic compounds, and surfactants are used chemical aids in environmental remediation. The structure of surfactants allows them to increase the aqueous solubility of nonpolar, hydrophobic compounds.

A surfactant molecule has a long, branched or unbranched, hydrocarbon tail, and a polar head (**Figure 2**). The hydrophobic tail has an affinity for nonpolar solvents, while the hydrophilic head has an affinity for polar solvents. The nonpolar portion of the surfactant is not compatible with water. Therefore, to minimize the free energy of the system, surfactants concentrate at the interfaces (air-water and/or surface-water). The hydrophobic portion faces either the air or the surface, that is, as distant from the

surrounding water as possible. Free surfactant can occur in solution only after these interfaces are saturated. In order to be thermodynamically favorable, these individual surfactant molecules aggregate into clusters called "micelles." The resulting spherical micelle contains a hydrophobic core, which appeases the hydrophobic tails, and a hydrophilic outer surface made of the polar head groups (Nakagawa, 1967). Hydrophobic compounds are able to partition into the core of the micelle resulting in improved solubilities (Laha and Luthy, 1991). The concentration in which the micelles first occur is termed the "critical micellar concentration," or CMC.

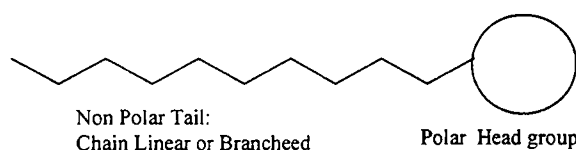


Figure 2: Diagram of a typical amphiphilic surfactant molecule (Muccini, 1997)

Surfactants are classified as anionic, cationic, and nonionic. These classifications are based on the electrical charge of the head group. These properties are important for application of remediation technologies. For example, nonionic surfactants tend to be less toxic than the cationic or anionic surfactants (Lewis, 1990). Also, nonionic surfactants are less likely to interact with charged soil particles.

Surfactant solutions can be used to wash soils contaminated with hydrophobic compounds. There are many benefits of soil washing. Surfactants can significantly increase the solubility of the hydrophobic contaminants and also mobilize contaminants in soil by lowering the capillary forces that hold them in soil pores. Thus, surfactants significantly improve the bioavailability of the contaminants for possible natural/induced biodegradation.

4.3 PCB Dechlorination by UV Irradiation

The toxicity of PCBs stem from chlorine substitution in the biphenyl molecule. Photolysis of these molecules were demonstrated in the early 1970s. The hexachloro biphenyl, 2,2',4,4',6,6' PCB, was photolyzed readily in organic solvents when UV-irradiated at 310 nm (Safe *et al.*, 1971). Other early photolysis experiments in aqueous and organic systems are also documented (Hutzinger *et al.*, 1974; Safe *et al.*, 1975). Since the aqueous solubility of PCB is low, mediums other than water have been used to solubilize PCBs for photodegradation studies.

Reductive dechlorination is the major pathway of PCB photodegradation (Huntzinger *et al.*, 1974). Upon photolysis of 4,4'PCB in *n*-hexane, only the parent compound and 4-PCB were found to remain in solution (Moore, 1985). Bunce performed photolysis of PCBs solubilized in an acetonitrile-water mixture (Moore, 1985) wherein the hydrogen atoms in the acetonitrile and water molecules were radio-labeled. After photolysis, 80-90% of the hydrogen atoms were abstracted from the acetonitrile, whereas only 10 –20%

of the hydrogen atoms were abstracted from water. This showed that nonpolar organic solvents were not only superior to water in solubilizing PCBs, they are also the preferred donors of hydrogen atoms in reductive dechlorination. Herring *et al.* (1972) found that Aroclor 1254 photodegraded faster in hexane than in water solutions. A later study of PCB photolysis in methanol obtained methanol substitution at the site in which the chlorine was lost (Moore, 1985).

Not all PCB congeners are photodegraded at the same rate. Studies with individual congeners and mixtures showed that congeners with more chlorine substitutions were degraded faster than those with fewer substitutions (Hutzinger *et al.*, 1972; Hannan *et al.*, 1973). Also, the position of the substituted chlorine affected the photodegradation rate. The quantum yield is usually low in compounds lacking *ortho* chlorine atoms but bearing *meta* or *para* chlorine atoms (Epling *et al.*, 1988). The efficiency of *ortho* chlorine loss was generally >0.1, whereas the efficiency of chlorine loss from other positions was typically around 1×10^{-3} (Bunce, 1982). Consequently, congeners with multiple substitution will lose chlorine atoms from the *ortho* positions relatively efficiently and subsequent chlorine loss will proceed at a much slower rate.

Photolysis of PCBs has the potential to form products that are more toxic than the original compound. Traces of 2-chlorodibenzofuran were identified from the photolysis of 2,5 and 2,2,5,5'-PCB (Moore, 1985). In another experiment, side reactions other than reductive dechlorination also produced more toxic dibenzofurans (Chu and Jafvert 1994; Epling *et al.*, 1988). To date, organic chemicals have been found to be superior to water

as solvents in PCB photolysis. However, toxic by-products, believed to form with organic solvents, could be potentially harmful.

4.3.1 Photolysis of PCBs in Surfactant Solutions

The main benefits of using surfactants in photolyzing PCBs are (1) increased solubility of PCBs, (2) increased quantum yield, and (3) attenuation of side reactions which produce toxic by-products (Chu and Jafvert, 1994). Epling *et al.* (1988) showed that photolyzed PCB solutions in the presence of sodium borohydride produced predominately dechlorinated products with little production of by products. Further, the photodegradation of PCBs was greatly accelerated by the presence of sodium borohydride (Epling *et al.*, 1988). In another study, photolysis of polychlorbenezes in micellar solutions of nonionic surfactants considerably increased the quantum yield compared to that in distilled water (Chu and Jafvert, 1994). The micelle interaction is important in photochemical reactions. The hydrophobic micellar core maintains much higher concentrations of the reactive species than would otherwise be in the homogeneous solution. Consequently, the reactive species in the micellar core has a much greater reaction opportunity which increases the probability of a reaction (Ramamurthy, 1986).

The efficiency of photochemical reactions is measured by dimensionless quantum yield defined as the number of moles of the light-absorbing substance that reacts for each mole of photon absorbed (Stumm and Morgan, 1996).

$$\Phi_\lambda = \text{(number of moles reacting/number of photons absorbed)}$$

Quantum yield can exceed unity because the absorption of a photon by a given compound may cause a chain reaction to occur and thus additional molecules may react (Schwarzenbach *et al.*, 1993). The proximity of the reactive species in the micellar core increases the probability of chain reaction.

4.4 Biodegradation of PCBs

PCBs were at one time thought to be too recalcitrant to be biodegraded. However, in the last twenty-five years, a large number of studies have shown that PCBs can be biodegraded aerobically, anaerobically, *in situ*, and *ex situ*.

Ahmed and Focht (1973) showed the first evidence of PCB biodegradation. The authors used two *Achromobacter* species to biodegrade several PCB. By the end of the 1970s, the scientific community generally accepted that lightly chlorinated PCB congeners (< 4 chlorine atoms) could be cometabolized whereas the highly chlorinated congeners were resistant to biodegradation (Unterman, 1996). During the 1980s and 1990s, many new PCB degrading bacterial strains were identified, new PCB degrading biological pathways were identified, and genetic engineering was used to improve PCB biodegradation (Unterman, 1996).

Anaerobic biodegradation of PCBs has been documented to occur in the natural environment, in the Hudson River sediments, for example (Brown *et al.*, 1987). Studies have shown that anaerobic PCB biodegradation occurs via reductive dechlorination (Quensen *et al.*, 1990). To its credit, anaerobic biodegradation preferentially removes the highly chlorinated congeners, making these compounds more soluble and vulnerable to aerobic biological attack. Anaerobic process also removes the *meta* and *para* chlorinated congeners rendering them less toxic to mammals (Unterman, 1996). But, anaerobic processes are extremely slow and may take from 8 to 16 weeks to yield satisfactory removal (Quensen *et al.*, 1990).

Aerobic PCB degradation generally occurs at faster rates and hence is more desirable for remediation. Major factors affecting aerobic PCB degradation are (Sylvestre, 1995):

Number of chlorine atoms and their positions on the molecule
Toxicity of the congeners
Biphenyl and the substrate/inducer
Bioavailability
Stringent control of metabolite production

The major pathway of aerobic PCB degradation involves the 2,3 dioxygenase attack (**Figure** 3). Through the use of four main enzymes (*bphABCD*), the biphenyl/chlorobiphenyl molecule is first hydroxylated and then one of the phenyl rings is cleaved between the 2 and 3 positions (Unterman, 1996). The presence of molecular oxygen is obligatory to the biochemical attack of the phenyl ring by *bphA*. In other words, oxygen is needed not only as an electron acceptor for respiration but also as a substrate for the dioxygenase enzyme attack. A second pathway was also isolated in

which the 3 and 4 positions on the phenyl ring were hydroxylated (Bedard *et al.*, 1987). **Figure 4** shows the two possible hydroxylation pathways by two different organisms.

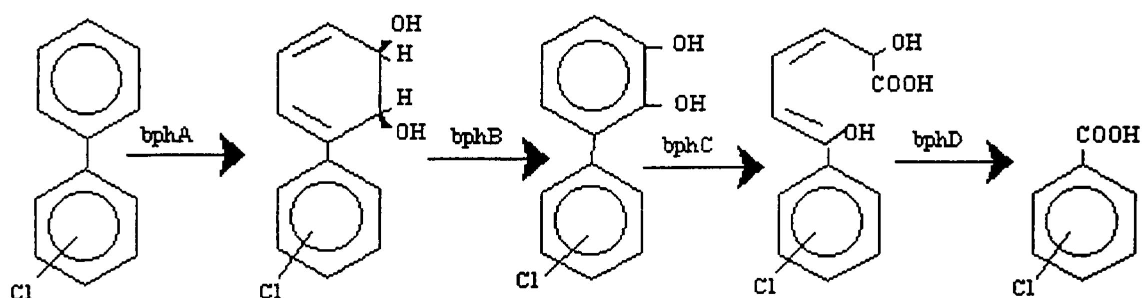


Figure 3. Degradation of chlorobiphenyl by the 2,3 dioxygenase pathway. Gene designation: *bphA* = biphenyl 2,3 dioxygenase; *bphB* = dihydrodiol dehydrogenase; *bphC* = 2,3 dihydroxybiphenyl dioxygenase; *bphD* = 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (meta cleavage product) hydrase (Muccini, 1997)

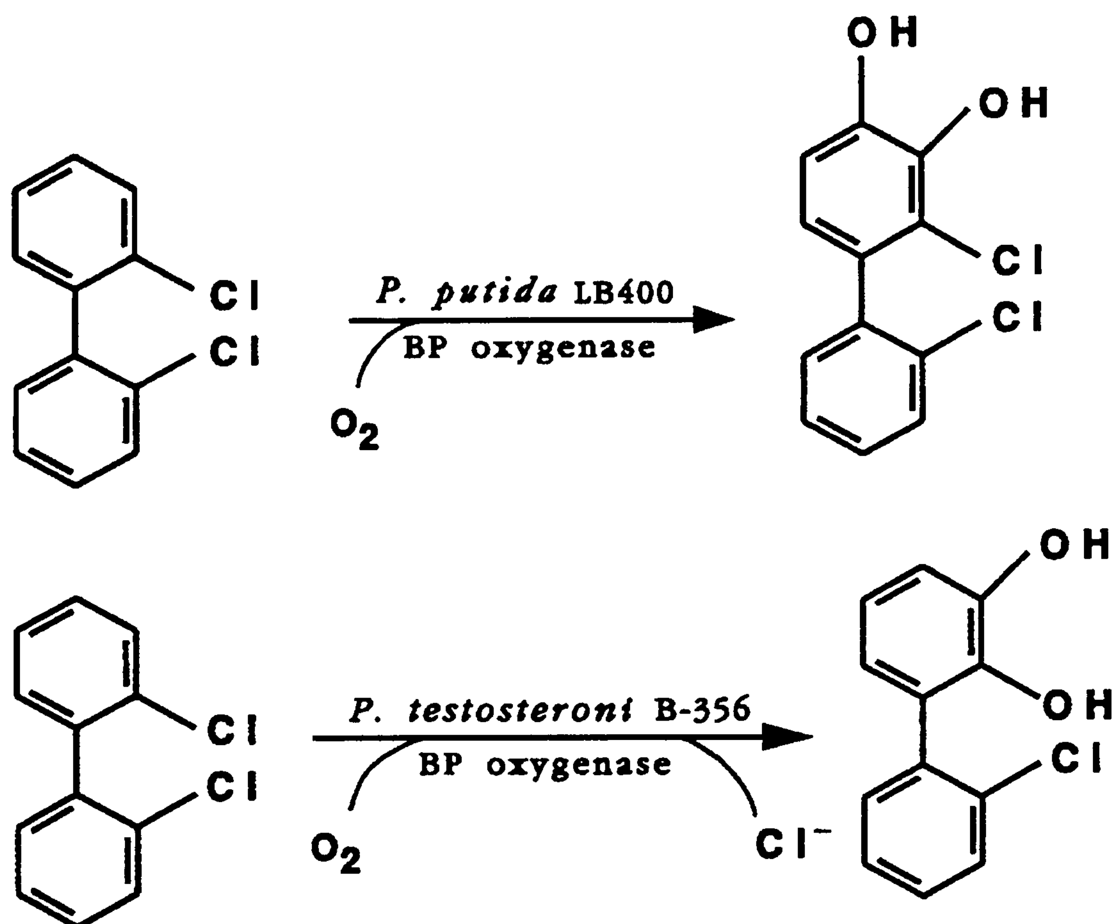


Figure 4. Two proposed pathways for initial oxygenase attack: 3, 4 dioxygenase (top) and 2,3 dioxygenase (bottom) (Sylvestre and Sondossi, 1994)

Few studies (if any) have determined the effect of oxygen concentration on the degradation of co-metabolites. However, there are a few studies that examined the effect of oxygen concentration on oxygenase enzymes. Shaler and Klečka estimated the half-saturation constant for oxygen of 1.2 mg/L during growth of a mixed culture on 2,4-dichlorophenoxyacetic acid (2,4-D) (1986). Degradation of 2,4-D was identical at O_2 concentrations between 2 mg/L to 8 mg/L. But it significantly decreased at O_2 concentrations less than 2.0 mg/L (measured within the reactor). In general, bacterial

growth rates are not affected above a critical dissolved oxygen concentration (Shaler and Klečka, 1986). The critical dissolved oxygen concentration is the concentration at which the respiration rate of the cells is one-half of the maximum rate observed at saturated levels (Shaler and Klečka, 1986). A variety of yeast and bacterial cultures are reported to have a critical dissolved oxygen concentration of 0.01 to 0.038 mg/L (Longmuir, 1954); some flocculant microbial cultures may require a minimum oxygen concentration of 0.5 mg/L for unhindered growth (Gaudy and Gaudy, 1980). Shaler and Klečka's study noted that microorganisms that use oxygen as a co-substrate for degradation of a chemical can be oxygen limited at a value much higher than the critical dissolved oxygen concentration needed for respiration. **Table 4** shows half-saturation constants for oxygen for a variety of organisms that use dioxygenase enzymes to degrade a specific substrate. All of these values are well above the reported critical dissolved oxygen concentrations.

Early studies reported that PCB congeners containing more than four chlorine atoms, or many *ortho* chlorine atoms, were resistant to aerobic biochemical attack (Furukawa *et al.*, 1978). However, in a recent study more than 90% degradation of highly chlorinated congeners and congeners with between 2 and 4 *ortho*-substituted chlorine atoms was obtained (Commandeur *et al.*, 1995). However, the influent concentrations of these congeners were extremely low, ranging from 0.4 to 25.3 μg/L. For an efficient, applied, bioremediation system, a much larger in-flow of PCBs would have to be attained.

Table 4: Half-saturation constants for oxygen for different organisms (Shaler and Klečka, 1986; Kroonen *et al.*, 1996)

Organism	Substrate	Oxygenase	Half-Saturation Constant Kdo (mg/L)	Reference
Pseudomonas arvilla C-1		Catechol-1,2-dioxygenase	0.6	Kojima *et al.*, 1967
Pseudomonas sp. OC1		Catechol-2,3-dioxygenase	0.3	Kojima *et al.*, 1961
Pseudomonas putida		3,4-Dihydroxy-phenylacetate-2,3-dioxygenase	0.8	Kita, 1965
Pseudomonas sp.		Melilotate-hydroxylase	1.6	Strickland and Massey, 1973
Pseudomonas putida		Orcinol-hydroxylase	2.2	Ohta *et al.*, 1975
Trichosporon cutaneum		Phenol-hydroxylase	1.7	Neujahr and Gaal, 1973
Pseudomonas aerugenosa		Protocatechuate-3,4-dioxygenase	1.4	Fujisawa and Hayaishi, 1968
Pseudomonas sp.		Protocatechuate-4,5-dioxygenase	1.7	Ono *et al.*, 1970
Pseudomonas acidovorans		Tryptophan-2,3-dioxygenase	1.1	Brady *et al.*, 1972
Pseudomonas aeruginosa JB2	2,5-dichlorbenzoate		1.0	Van der Woude *et al.*, 1995
Pseudomonas aeruginosa JB2 variety 2	2,5-dichlorbenzoate		1.0	Van der Woude *et al.*, 1995
Pseudomonas sp. strain A3	3CBA (3-chlorbenzoate)		0.8	Kroonmen *et al.*, 1996
Pseudomonas sp. strain H1	Benzoate		3.52	Haller and Finn, 1979
	Benzoate		10.0	Haller and Finn, 1979
Mycobacterium sp.	Pyrene		0.2	Fritzsche, 1994
Enrichment	2,4-dichlorophenoxyacetic acid		1.2	
Pseudomonas putida	Catechol		2.6	Shaler and Klečka, 1986
Alcaligenes sp. strain L6	3CBA			Viliesid and Lilly, 1992
	With O_2 excess		0.5	Kroonmen *et al.*, 1996
	With O_2 limitation		0.3	Kroonmen *et al.*, 1996

4.4.1 Biodegradation of PCBs in Surfactant Solutions

Surfactants are used to increase the solubility and thus the bioavailability of hydrophobic contaminants. Studies show variations in biological activities in surfactant/contaminant solutions. Microorganisms can biodegrade contaminants without degrading the surfactant (Liu, 1980); microbes can biodegrade both the contaminant and surfactant (Tiehm *et al.*, 1997); and microbes can biodegrade only the surfactant while fortuitously degrading the contaminant (Lajoie *et al.*, 1994). The surfactant may be toxic to microorganisms (Auger *et al.*, 1995) or surfactant micelles may interfere with cellular metabolism of contaminants (Auger *et al.*, 1995). Also, whereas a number of researchers observed that contaminants partitioned into the micelles are bioavailable (Volkering et al., 1995; Guha and Jaffe 1996b), other researches have found that these partitioned contaminants are not readily bioavailabile (Laha and Luthy, 1991; Guha and Jaffe, 1996b). On the other hand, easily biodegradable surfactants may lead to a depletion of oxygen (Tiehm *et al.*, 1997). Many nonionic surfactants are not toxic towards microorganisms; however, some of these nonionic surfactants inhibit bacterial biodegradation of polyaromatic hydrocarbons (PAHs) (Guha and Jaffe, 1996a). Polyoxyethylene 10 lauryl ether (POL (10)) did not inhibit phenanthrene degradation in batch cultures at up to 4 times its CMC (Guha and Jaffe, 1996a).

Field application vectors (FAVs) were developed at The University of Tennessee to address the problems of surfactant toxicity and PCB bioavailability (Lajoie *et al.*, 1994). FAVs were originally developed to allow the expression of foreign genes in nonsterile competitive environments (Lajoie *et al.*, 1993). Two PCB degrading FAVs,

Polyoxyethylene 10 lauryl ether

P. putida IPL5::TnPCB

dodecyloxy)ethanol

A. eutrophus B30P4::TnPCB

A. eutrophus B30P4::TnPCB

≅ 90% Degradation

Figure 5. Degradation of POL (10) by IPL5::TnPCB and B30P4::TnPCB (Lajoie *et* al., 1997)

Psuedomonas putida IPL5::TnPCB and *Ralstonia eutropha* B30P4::TnPCB, were created by inserting the genes for biphenyl/PCB degradation via a transposon into the chromosome of surfactant degrading bacteria (Lajoie *et al.*, 1994; Lajoie *et al.*, 1997). When used in combination, these strains are capable of degrading near 90% of POL (10),

without the production of detectable products, and 70% of the PCBs (**Figure 5**) (Lajoie *et al.*, 1997).

4.5 Sequential Photochemical and Biochemical Degradation of PCBs

Only a limited number of studies explored the effects of both photochemical and biochemical degradation of PCBs. Baxter and Sullivan (1984) suggested that photochemical reactions in the environment degrade PCB compounds that were already biochemically transformed by microbial oxidation. Kong and Sayler (1983) concluded that irradiation altered the parent 4-chlorbiphenyl molecule (or its metabolites) so that it was more susceptible to microbial mineralization. Shimura *et* al. (1996) examined the use of photolysis prior to biodegradation. The authors irradiated 2-mL of 500 mg/L Kaneclor 500 (KC500) in a methanol solution for 26 hours. The KC500 was then concentrated to 125 mg/L, and the remaining KC500 was completely degraded by *Psuedomonas alcaligenes* TK102 after one week.

5. MATERIALS AND METHODS

5.1 Bacterial Cultures

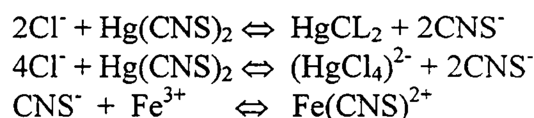
Four bacterial strains, namely used were *Pseudomonas putida* IPL5::TnPCB, *Ralstonia eutropha* B30P4::TnPCB, *Psuedomonas putida* IPL5::TnTc, and *Ralstonia eutropha* B30P4::TnTc, were used in this study. To make stock cultures, each bacterial strain was grown individually in a medium containing 12.5 μg/μL tetracycline, 0.2% surfactant, and 25 mL PAS minimal salts medium (Bopp, 1986) at 25°C. The PAS minimal salts media was made by combining 911 mL of de-ionized (DI) water, 78 mL PA concentrate, 10 mL of PA salts, and 1 mL of 5% yeast extract. The PA concentrate solution was made with 56.8g K_2HPO_4, 22.0g KH_2PO_4, and 27.0 g NH_4Cl dissolved in DI water and made up to 1-L which was then autoclaved. The PA salts solution was made by dissolving 19.5g $MgSO_4$, 5g $MnSO_4 \bullet H_2O$, 1g $FeSO_4$, and 0.3g $CaCl_2 \bullet H_2O$ in 1-L of DI water and then adjusted to pH 2.5 with H_2SO_4, filtered and sterilized. These cultures were grown to an optical density (OD) of 1.0. Equal volumes of the two culture and a 40% glycerol solution were mixed, and 1 mL volumes were dispensed into cryogenic vials. These tubes were placed in a –80°C freezer. Whenever culture was needed for an experiment, one vial was taken from each bacterial strain and combined with the necessary growth requirements.

5.2 Photolysis

All photolysis experiments were done using a 210 mg/L solution of Aroclor 1242 dissolved in 4 g/L POL (10). The PCB solution was placed into a quartz tube (id x length = 4 cm x 32 cm). The solution was kept well mixed using a small, teflon-coated magnetic stirrer bar placed inside the quartz tube. The quartz tube was suspended in the middle of a Rayonet photoreactor (Model #97053) which contained 16 low pressure UV lamps. The maximum UV light output was 9.59×10^{-5} E m^{-2} sec^{-1} at 254 nm. In different experiments, the samples were photolyzed for periods of up to 300 minutes.

5.3 Chloride Measurements (Colorimetric)

Dechlorination of PCB was monitored by measuring the appearance of chloride ion in solution as photolysis progressed. A colorimetric method was used for chloride analysis (Iwasaki *et al.*, 1952). The chemistry for the formation of the colored complex, $Fe(CNS)^{2+}$, is given below:



$$2Cl^- + Hg(CNS)_2 \Leftrightarrow HgCL_2 + 2CNS^-$$
$$4Cl^- + Hg(CNS)_2 \Leftrightarrow (HgCl_4)^{2-} + 2CNS^-$$
$$CNS^- + Fe^{3+} \Leftrightarrow Fe(CNS)^{2+}$$

A mercuric thiocyanate solution was made by dissolving 0.3g of mercuric thiocyanate in 100 ml of 95% ethyl alcohol. A ferric alum solution was prepared by dissolving 6g of ferric ammonium sulfate in 100 mL of 6N nitric acid. A series of standard potassium

chloride (KCl) solutions were made. To prepare a standard calibration curve, to 10 mL of each standard KCl solution 1 mL of the mercuric thiocyanate solution and 2 mL of the ferric alum solution were added. Following color development, 1 mL of this solution was placed in a quartz cuvette, and absorbance measured at a wavelength of 460 nm. A standard curve for Cl^- concentration in mg/L versus absorbance was produced. The absorbance in photolyzed PCB solutions were measured in the same way and the chloride concentration determined using the calibration curve.

5.4 Electrochemical Method for Chloride Measurement

Chloride concentration in solution was also measured electrochemically using a chloride selective electrode (Model Orion 96-17). Using standard KCl solutions, a standard curve was produced correlating chloride concentration (mg/L) with EMF in millivolts (mV). At selected times during photolysis, 3-4 mL of test solution was withdrawn and EMF measurements were made using the chloride electrode. Chloride concentration in mg/L was determined using the standard curve.

5.5 Biodegradation Studies

The biodegradation experiments were done in batch reactors consisting of 1-L Ehrlemeyer flasks fitted with rubber stoppers with two holes in each through which 1-mL glass pipettes were inserted. One of the pipettes was connected by rubber tubing to an air pump and an air flow regulator while the other served as a vent. An air flow of 750

cm^3/minute was used for the first four days of growth and a flow of 250 cm^3/min was used thereafter. Each reactor contained 300 mL of the biological reaction mixture kept well mixed using a 3-inch magnetic stir bar and a magnetic stir plate.

The 300 mL of biological solution consisted of 0.3 mL yeast extract, 3 mL (PAS) salts, 23.4 mL PA concentrate, 243 mL of POL 10 surfactant solution, deioized water, and PCBs, and 15 mL each of the stock cultures of *Psuedomonas putida* IPL5::TnPCB and *Ralstonia eutropha* B30P4::TnPCB were used in the experimental reactor. The two surfactant-degrading bacteria were found to degrade more of the surfactant jointly rather than individually (Lajoie *et* al., 1997). Further, these strains contain a transposon containing genes that code for PCB degrading enzymes. To avoid potential problems in PCB mass-balance arising from adsorption and volatilization losses (Vrana *et al.*, 1996; Dercova *et al.*, 1996), separate control bioreactor was operated in parallel. The control reactor contained a similar mixture of bacteria, *Psuedomonas putida* IPL5::TnTC and *Ralstonia eutropha* B30P4::TnTC with the transposon that encodes for the PCB-degrading enzymes missing. The difference in PCB concentrations in the two reactors represented net biodegradation at agiven time. The abiotic losses include loss due to adsorption on reactor walls, loss due to precipitation of PCBs when surfactant concentration in solution fell below CMC because of biodegradation, and volatilization, among others.

Cumulative PCB mass biodegraded in a given time period was calculated from the difference in net (control - experimental) PCB concentrations at the beginning and the

end of the duration multiplied by the reactor volume. Percent PCB biodegradation can be expressed as follows:

$$\text{PCB Biodegradation (\%)} = [1-(PCB_{ex}/PCB_{con})]*100$$

Where, PCB_{ex} = PCB concentration in the experimental reactor

PCB_{con} = PCB concentration in the control reactor

5.6 Assay of Estrogenic Activity

The yeast growth inhibition assay (Routledge and Sumpter, 1996) was used to determine if PCBs, and/or the by-products of aerobic PCB metabolism demonstrated any estrogenic activity. A yeast stock culture was prepared by inoculating a growth medium with 125 µL of 10X concentrated yeast stock. This was incubated for 24 hours at 28°C on an orbital shaker. In a type II laminar air flow cabinet, the chemicals to be assayed were serially diluted with absolute ethanol. Ten microliter aliquots of each concentration were transferred into a 96-well optically flat bottom microtitre plate. Ten microliters of absolute ethanol was added to the blank wells. The chemicals in the assay plate were left to evaporate until completely dry. 200 µL of the seeded assay medium (growth medium containing chlorophenol red-B-D-galactopyranoside (CPRG) and yeast) was added to the wells. This 1/20 dilution gave a final concentration of chemicals of (100 mg/L to 50 µg/L) and 17β-estradiol of 2,724 ng/L (1×10^{-8}M) to 1.36 ng/L (5.0×10^{-12} M). A standard curve (absorbance vs. known 17β-estradiol concentration) was obtained for each assay. The plates were sealed with autoclave tape and were vigorously shaken for 2 min on a titre plate shaker. Next, the plates were incubated at 32°C for three days and each

day the vigorous shaking was repeated. Finally, the absorbance of each samples was measured at 540 nm and 620 nm using a plate reader (spectrophotometer capable of reading 96-well plates) for color and turbidity, respectively. The color indicated the presence of estrogen, and the lack of turbidity, as compared with a control, indicated growth inhibition.

5.7 Assay for Cell Growth

The growth of cells in the bioreactor was measured using two methods: cell protein assy and optical density (OD). Protein was measured using the Pierce BCA Protein Assay Kit. A 50:1 ratio of Pierce solutions A and B were mixed to create a working solution. 100 µL of sample was placed into a borosilicate glass test tube (Fisher) with 2 mL of the working solution. The samples were incubated at 37°C for 30 minutes, cooled for 5 minutes, and then the absorbance was measured at a wavelength of 562 nm (Pierce Protocol for the BCA Protein Assay Reagent, product No. 23225) using a spectrophotometer. The OD of the growing culture was measured at a wavelength of 600 nm relative to that of water (blank).

5.8 Assay of 2,3 Dihydroxybiphenyl Dioxygenase Activity

The activity of 2,3 dihydroxybiphenyl dioxygenase enzyme, encoded by the bphC gene, was measured as a function of time using whole cells. A solution of 0.11 mM 2,3 dihydroxybiphenyl was made in a 50mM Na/K buffer. A 100µL whole cell sample from

the bioreactor was injected into a polypropylene cuvette containing 0.9 mL of the buffered 2,3 dihydroxybiphenyl solution. The blank consisted of 100 μL of whole cell sample injected into 0.9 mL of the buffer solution with no 2,3 dihydroxybiphenyl. The absorbance (A) of the sample was measured at a wavelength of 434 nm against the blank and plotted as a function of time. An extinction coefficient (ε) of 221 $mM^{-1}cm^{-1}$ (Kuhm *et al.*, 1991) was used to calculate concentration ($c = A / \varepsilon$) of the enzyme. The initial slope ($\Delta A/\Delta t$) of the absorbance-time plot was taken as a measure of the rate of change in enzyme concentration. These initial slopes were taken for varying times during the biodegradation experiments.

5.9 pH

Five milliliter samples were placed in scintillation vials and the pH was measured using an Orion 720A pH meter. The pH was adjusted to 7.0 with 1 M NaOH as necessary. Up to 10.6 mL of 1 M NaOH was used to adjust pH of the bioreactor mixture (300 mL) or about 3.5% (v/v) of the reaction volume.

5.10 Dissolved Oxygen

Dissolved oxygen (DO) concentration in the bioreactor was electrochemically measured using a YSI oxygen meter (Model 5000) and a YSI oxygen probe (Model 5739). First, the ambient atmospheric pressure value was entered into the oxygen meter. The rubber stopper from the bioreactor flask was removed and the DO probe was carefully immersed

into the solution without touching the bottom or wall of the flask. The DO meter was allowed to stabilize before duplicate measurements.

5.11 Intrinsic Oxygen Uptake Rate

A YSI respirometer (Model 5301) was used to measure the intrinsic oxygen uptake rate during biodegradation. Three milliliters of solution was removed from the bioreactor and placed into a glass vial. This glass vial contained a magnetic stir bar, and both the vial and the stir bar set onto a YSI 5301 apparatus. The solution was stirred for 5 minutes to ensure saturation with oxygen. Then, a YSI oxygen probe (Model 5331) was inserted into the glass vial. Care was taken to ensure that absolutely no air bubbles remained in the solution. The decrease in DO concentration continuously measured under quiescent conditions and recorded by a X-Y plotter (Perkin-Elmer 023). The slope of the linear region of this graph represented the rate of oxygen uptake. Dividing the slope by the weight of total cell protein yielded an estimate of specific oxygen uptake rate (SOUR).

5.12 Preparation and Analysis of PCB Stock Solution

PCB Solubilization: Aroclor 1242, the commercial PCB used in this study, has a solubility of only 200 μg/L (Huntzinger et al., 1974). Surfactant solutions of Aroclor 1242 were prepared by adding excess Aroclor 1242 to a known amount of POL (10). These solutions were mixed for 3 days in an end-over-end rotator (Wheaton) and centrifuged at 10,000 rpm for 30 minutes. The surfactant/PCB solutions were siphoned,

leaving excess (undissolved) Aroclor 1242 behind. Siphoned solutions were used as stock solutions of Aroclor 1242 in POL (10) for photolysis, biodegradation, and solubility experiments.

PCB Analysis by Gas Chromatography: To prepare PCB solutions for analysis by gas chromatography (GC), the samples were extracted in triplicate with hexane. One milliliter aliquots were added to 0.3 g of a silica based powder, tC_{18} (Waters, Inc.) in 15 mL glass tubes. Eight milliliters of hexane was then added, and the contents shaken for 8 hrs on a horizontal shaker. After a centrifugation for 5 minutes at 1000 rpm, a sample was taken using a syringe (Hamilton) and further diluted with hexane in a GC vial, and then capped with a Teflon lined cap. The PCBs in the hexane phase were analyzed using a gas chromatograph (Shimadzu: Model GC-14A) equipped with an AOC-14 autoinjector, an electron capture detector (ECD), and a DB-1 capillary column (id x length: 0.25 mm x 30 m) (J & W Scientific, Inc.) with Nitrogen as the carrier gas. The column oven temperature was held at 60°C for 2 min, raised to 180°C at a rate of 10°C/min, raised to 225°C at a rate of 6°C/min, held at 225°C for 14 min, raised to 280°C at a rate of 5°C/min, and then held at 280°C for 13.5 min (Bedard, 1987). The PCB concentrations were compared to an Aroclor 1242 standard (Ultra Scientific, Inc). Individual congeners were identified and quantified to obtain the total PCB concentration (Frame *et al.*, 1996). The total PCB concentrations were normalized using peak number 41 (the non-biodegraded congeners 2,3,4,3',4' and 2,3,4,2',3',5') as an internal standard for the biological samples. For the photolysis samples, 5 μL of 2,2',3,3',4,5,6,6'-

octachlorobiphenyl (100 mg/L) was added into the GC vial directly and this congener was used as the internal standard.

5.13 Surfactant Measurements

POL (10) was quantitatively analyzed using the cobaltothiocyanate active substance method (CTAS) (American Public Health Association, 1989 Standard Methods). Triplicate 1.5 mL samples from the bioreactor were centrifuged at 11,000 rpm for 5 minutes in eppendorf tubes. One milliliter of the supernatant was added to 3 mL of the cobal thiocyanate reagent (36g $Co(NO_3)\bullet 6H_20$, 240 g NH_4SCN/L H_20) and 8 mL of methylene chloride into 15 mL centrifuge tubes. These tubes were shaken for 10 minutes in a horizontal shaker and then centrifuged at 1000 rpm for 5 minutes. 1 mL aliquot of the methylene chloride phase was placed in a quartz cuvette and its absorbance measured at a wavelength of at 620 nm using a spectrophotometer. Absorbance was related to POL (10) concentration using a standard curve.

6. RESULTS AND DISCUSSION

6.1 Surfactant Solubilization of PCBs

The objective of this section was to examine how surfactants enhance the solubilization of PCBs. The reported aqueous solubility of Aroclor 1242 is 200 μg/L (Hutzinger *et* al., 1974). Surfactants may be used to increase aqueous solubilility of PCBs at dosages above the CMC. The reported CMC of POL (10) is 1 x 10^{-4} M (63 mg/L) (Yeom *et* al., 1996). Solubility measurements with Aroclor 1242 in POL (10) solutions increased linearly at concentrations above the CMC (**Figure 6**). Other sources have reported similar linear relationships between surfactant and hydrophobic contaminant concentrations (Jahan *et* al., 1997). The solubility (at saturation equilibrium) of Aroclor 1242 in 4 g/L POL (10) was approximately 920 mg/L. The molar solubility ratio (MSR) for the dissolution of Aroclor 1242 in POL (10) was determined from the slope of the straight line above the CMC (Edwards *et* al., 1991). MSR represents the ratio of the number of moles PCBs dissolved per mole of POL (10). The molecular weight of Aroclor 1242 was calculated to

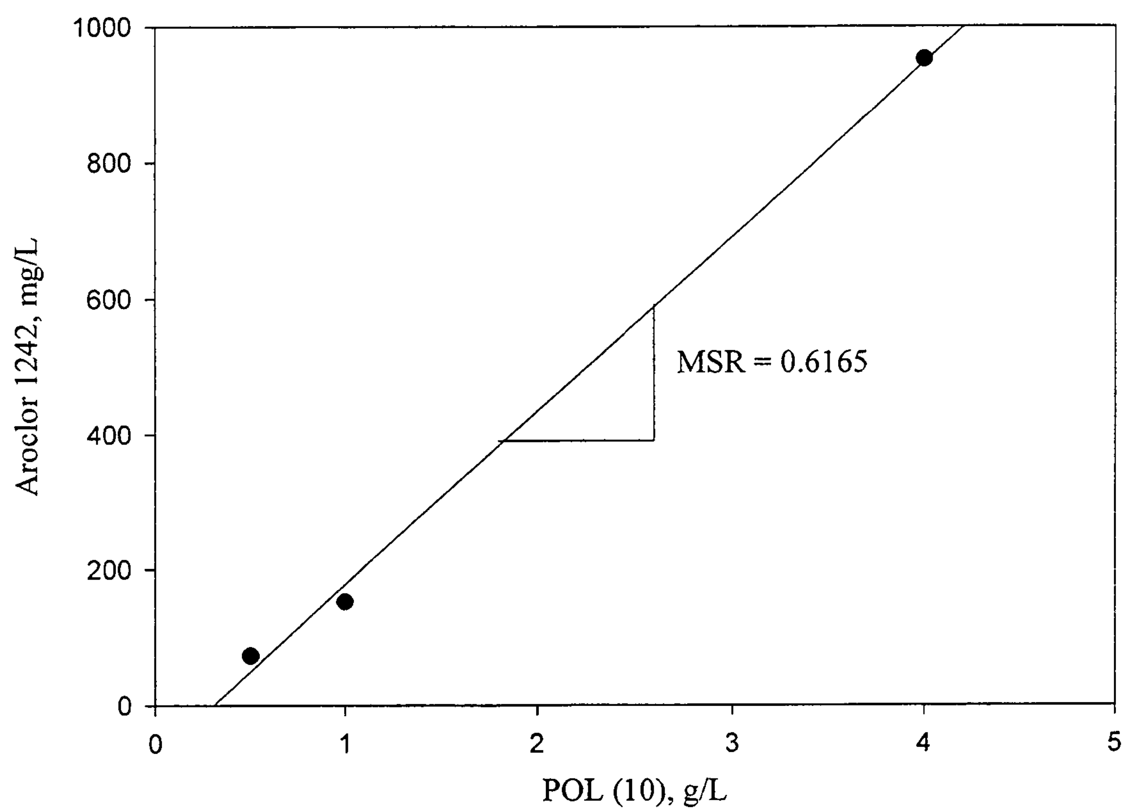


Figure 6. Solubility of Aroclor 1242 in POL (10).

be 260.3 g/mol using weighted molecular weights of the various congeners. This molecular weight was used to determine MSR given by Equation 1. The MSR of Aroclor 1242 in POL (10) was 0.6165. The MSR value allows comparison of solubilities of a hydrophobic compound in different surfactants. As shown in Equation 2, the micellar partitioning coefficient, K_m, was determined using the MSR value (**Table 5**).

$$MSR = (S - S_{cmc})/(C - CMC) \quad (1)$$

$$K_m = X_m/X_a$$

$$= MSR/[(MSR + 1)(S_{cmc} V_w)] \quad (2)$$

where, X_m = mole fraction of PCB in micellar pseudophase

X_a = mole fraction of PCB in aqueous pseudophase

S = solubility of PCB at $C > CMC$, (M)

S_{cmc} = solubility of PCB at CMC, (M)

C = surfactant concentration at which S is measured, (M)

V_w = molar volume of water = 0.0181 M^{-1} at 25°C

Table 5: Characteristics of Aroclor 1242-POL (10) System

PCBs	**Molecular Weight of the PCBs. g/mol**	**Surfactant**	**MSR**	**Log K_m**	**CMC of POL (10), mol/L**
Aroclor 1242	260.3	POL (10)	0.6165	7.44	8.39×10^{-5}

Figure 7 shows a typical gas chromatogram of Aroclor 1242 dissolved in POL (10). The amounts of the various congeners in solution can be calculated from the gas chromatograms. Similar gas chromatograms were used to visually compare the progress

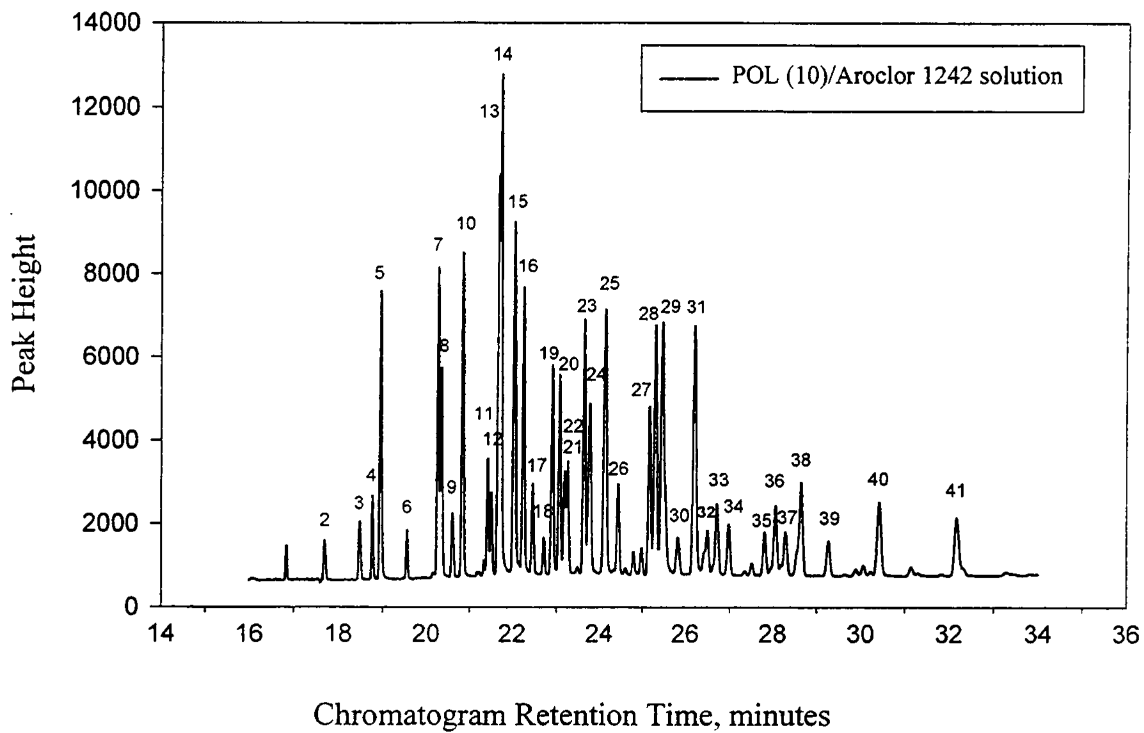


Figure 7. Typical gas chromatogram of Aroclor 1242 in POL (10) solution (the numbers on the peaks represent the peak numbers).

of PCB biodegradation and are presented in later to demonstrate the differences in PCB degradation based on the type and duration of various treatment modes.

6.2 Photolysis

To determine if photolysis improved biodegradation of PCBs, surfactant-solubilized Aroclor 1242 was UV-irradiated at a wavelength of 254 nm. Highly chlorinated PCB

congeners (more than 4 chlorine atoms per biphenyl molecule) have been shown to be recalcitrant to biodegradation by the aerobic PCB-GEMs. Photolysis can be employed to reductively dechlorinate PCBs (Huntzinger *et al.*, 1974). A suite of photolysis experiments was conducted to determine the optimum length of irradiation preceding biodegradation in an effort to maximize the degradability of PCB.

A 210 mg/L Aroclor 1242 in 4 g/L POL (10) was photolyzed for 300 minutes. These concentrations of PCBs and surfactant were selected to mimic a realistic waste stream following surfactant-washing of contaminated soils. Samples were collected at various times during photolysis, 0, 2, 5, 10, 20, 40, 60, 90, 120, 180, 300 minutes, and analyzed for surfactant, PCB, and chloride ion concentrations (**Figure 8**).

POL (10) concentration remained constant (+/- 10%) throughout the experiment, so that all of the Aroclor remained in the micellar pseudophase and was available for solubilization of the PCBs. By the end of the 5-hr experiment, only 3.5% of the surfactant was degraded.

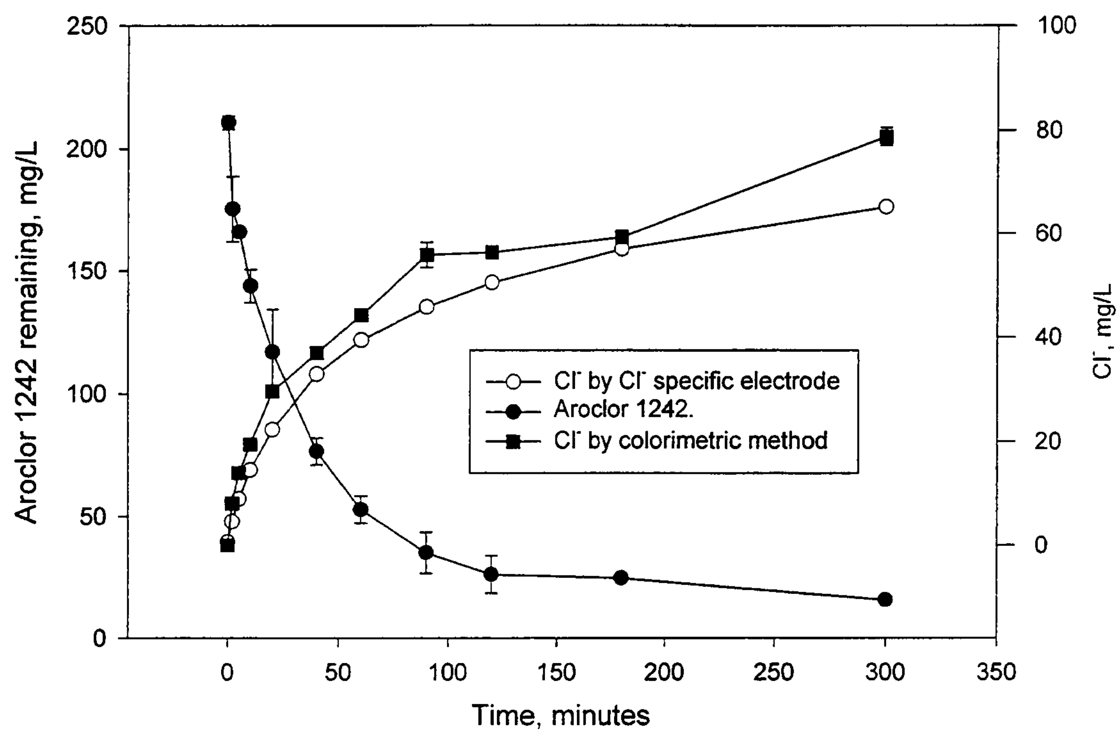
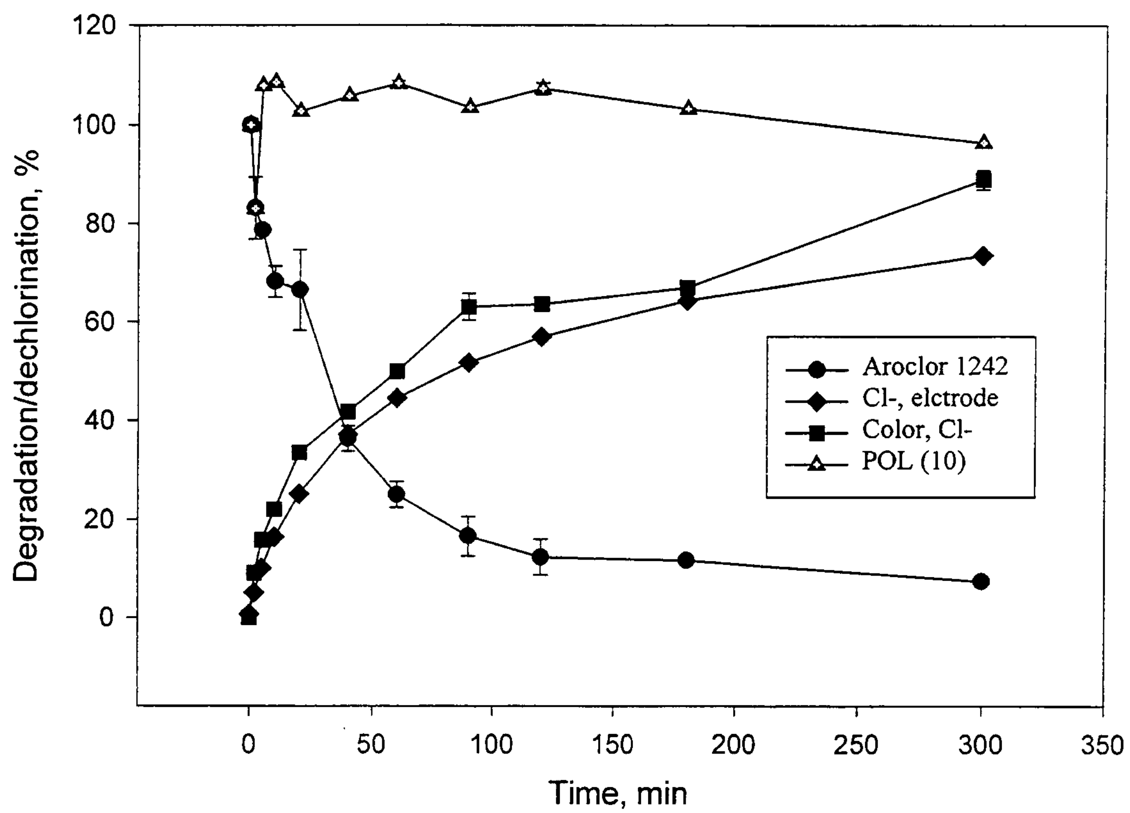


Figure 8. Aroclor 1242 and chloride concentrations during photolysis. (A) concentrations in mg/L



(B) Percent photodegradation and dechlorination percentage as a function of time

Both the initial PCB degradation and the dechlorination rate followed first order kinetics for the first 10 minutes of irradiation. Thereafter, absorption of photons by the by-products caused a deviation from the first-order kinetics. The first-order rate constant, k_p, during the initial period was 6.374×10^{-4} sec^{-1}. The initial dechlorination rate constant, k_p, was determined to be 4.145×10^{-4} sec^{-1}. This dechlorination rate constant, calculated from the change in Cl^- ion measured colorimetrically was about 65% of the degradation rate or 4.14×10^{-4} sec^{-1} method; however, the electrochemical method gave a value of 2.983×10^{-4} sec^{-1}, slightly lower than that obtained colorimetrically. Also, 90% of the initial Aroclor 1242 mass was accounted for based on the measured PCB remaining in solution and the mass of PCB degraded calculated from the measured Cl^- mass appearing in solution. The excellent correspondence between the two rates and the observed mass balance indicates reductive dechlorination to be of the major pathway of the photodegradation of PCBs. Previous experiments using PCBs in organic solvents also noted dechlorination to be the primary pathway of photodegradation (Sawhney, 1986; Hutzinger *et al.*, 1974).

The quantum yields given in **Table 6** were calculated using Equation 3 (Zepp, 1978):

$$M = k_p C / [I_\lambda (A/V)] \quad (3)$$

where, I_λ = flux of incident light of a specific wavelength

$I_\lambda = 9.59 \times 10^{-5}$ E m^{-2} sec^{-1} at 8 = 254 nm

C = concentration of Aroclor 1242 = 8.09×10^{-4} (M)

k_p = first order rate constant (initial 10 min of photolysis) (sec^{-1})

A/V = (exposed area : volume of sample) = 102.6 m^{-1}

Table 6: Initial ($\leq$ 10min) photolysis rate and quantum yield for 210 mg/L Aroclor 1242 in 4 g/L POL (10) solution

	Rate, k_p (sec^{-1})	**Quantum Yield, M**
Degradation	6.374×10^{-4}	*4.740×10^{-5}*
Dechlorination	4.145×10^{-4}	*3.257×10^{-5}*

The concentrations of total PCB and specific congeners varied over time (**Figure 9**). Highly chlorinated congeners (retention time > 33 min) were completely removed in 300 minutes. The progressive dechlorination of congeners with large number of chlorine substitution is well illustrated by the changing congener profiles depicted in these figures. The persistence of certain low molecular weight congeners even after 300 minutes may be attributed to the reductive dechlorination of highly chlorinated congeners.

There may be some correlation between the amount of chlorine on the congener and the rate of photodegradation. It has been documented that the more chlorine on the congener, the faster the rate of photodegradation (Huntzinger *et al.*, 1974; Hannan et al., 1973).

The goal of photolysis is to maximize the degradation of the non-biodegradable peaks while minimizing the time of photolysis. Based on the chromatograms of photolyzed Aroclor 1242 (**Figures 9**), forty minutes was determined to be the optimum duration of photolysis as a pretreatment for aerobic biodegradation. Highly chlorinated congeners

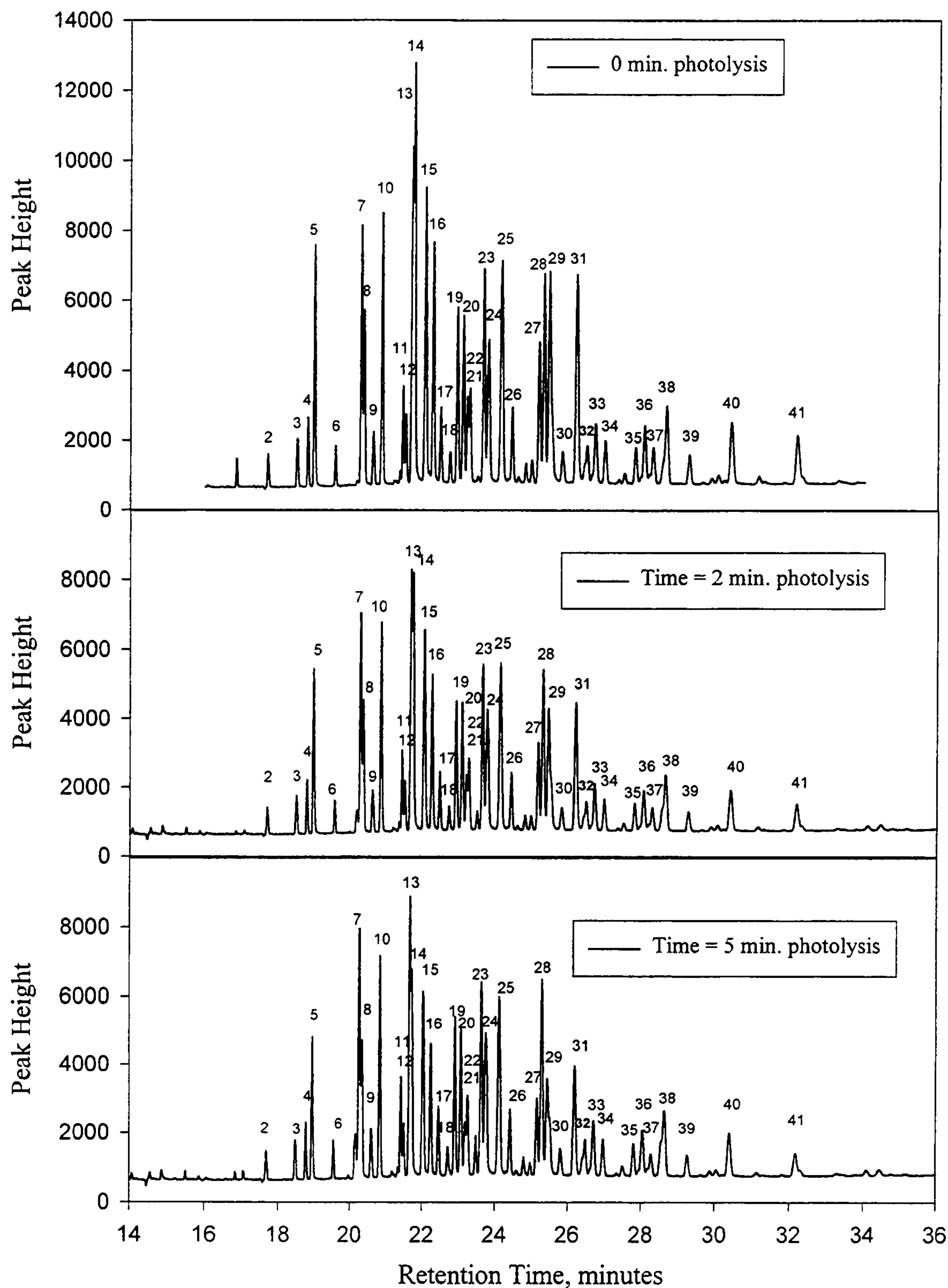
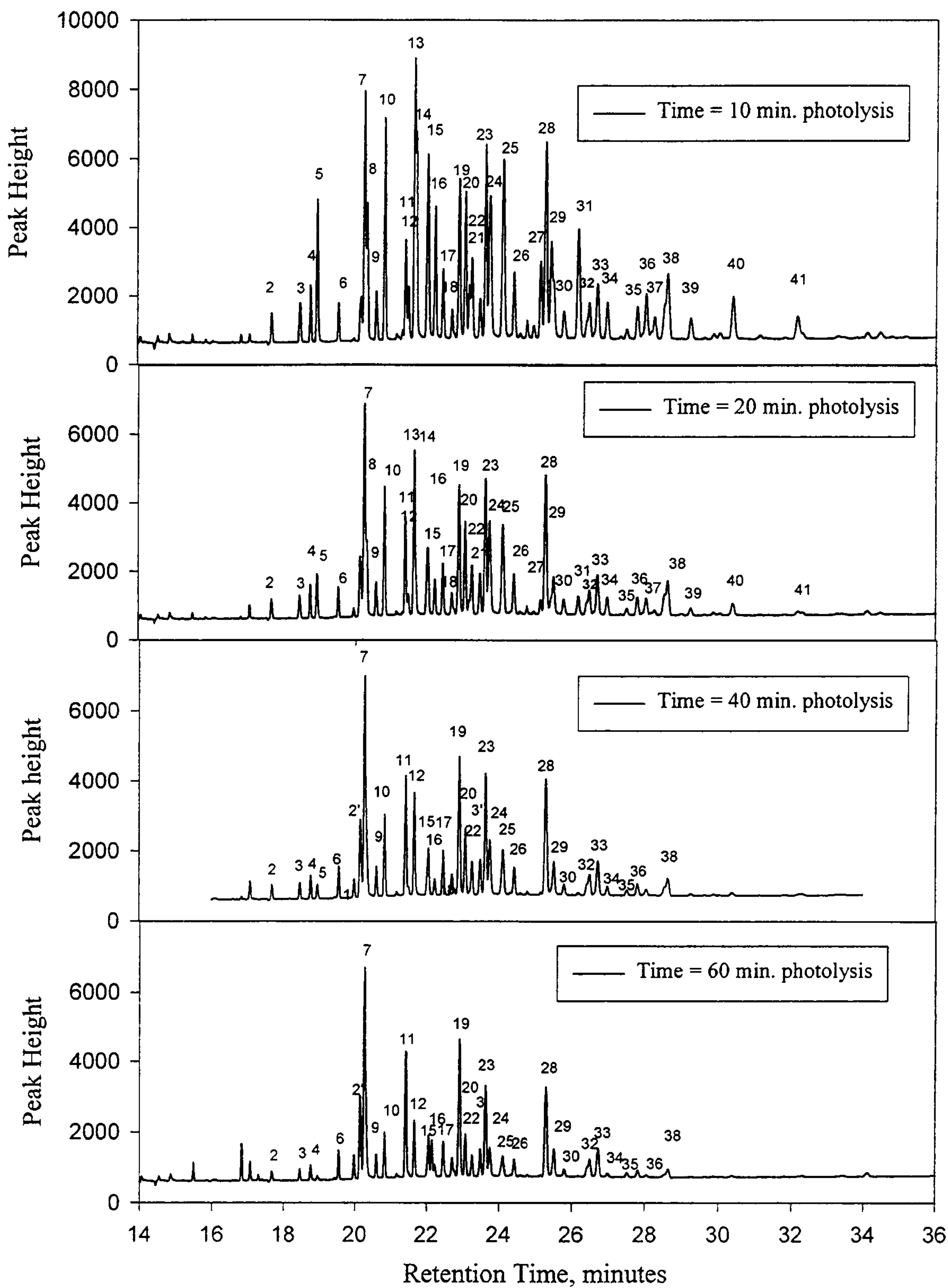
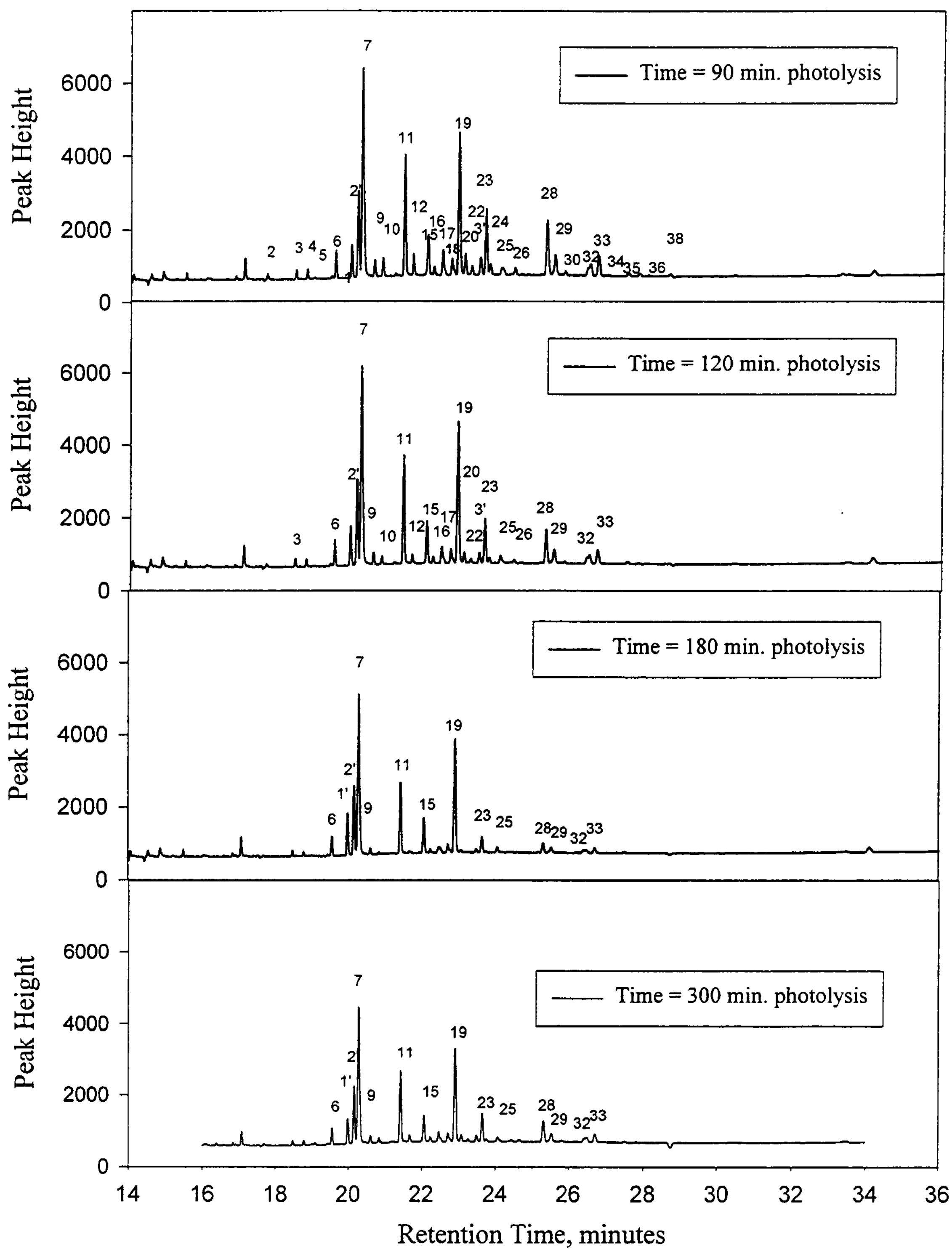


Figure 9. Photolysis of Aroclor 1242 in POL (10) solution. (A) 0 to 5 minute durations



(B) 10 to 60 minute durations



(C) 90 to 300 minute durations

were largely destroyed after 40 minutes leaving a large portion of the lightly chlorinated congeners to be biologically degraded.

6.3 Biodegradation Studies

6.3.1 Biodegradation of POL(10)

In previous experiments, the PCB-GEMs were shown to metabolize POL (10) and concurrently co-metabolize PCBs (Lajoie *et al.*, 1997). To determine the effect of PCBs on the biodegradation of POL (10) by the PCB-GEMs, biodegrdation of POL (10) was studied with and without PCBs. The degradation of 10 g/L and 2 g/L POL (10) in the presence of 50 mg/L of Aroclor 1242 and with no PCBs are shown in **Figure 10**. The PCB-GEMs seemed to metabolize POL (10) equally well in solutions with and without PCBs. Also, optical densities of the PCB-GEMs were similar regardless of the presence of PCBs (**Figure 11**). Further, the specific substrate utilization rate of POL (10), q, was nearly equal with and without PCBs (**Table 7**). The specific substrate utilization rate was calculated by:

$$q = (dS/dt)/X$$

where, q = specific substrate utilization rate, hr^{-1}

dS = change in substrate concentration, g/L POL (10)

dt = change in time, hr

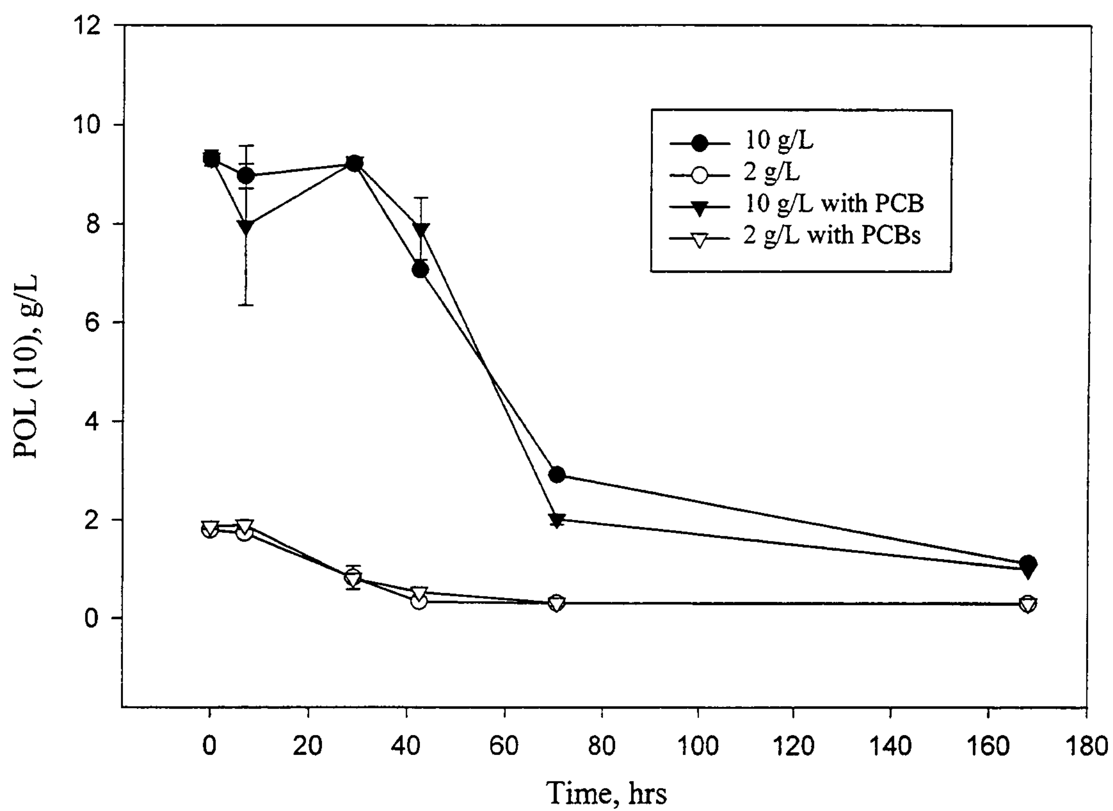


Figure 10. POL (10) biodegradation with and without 50 mg/L Aroclor 1242

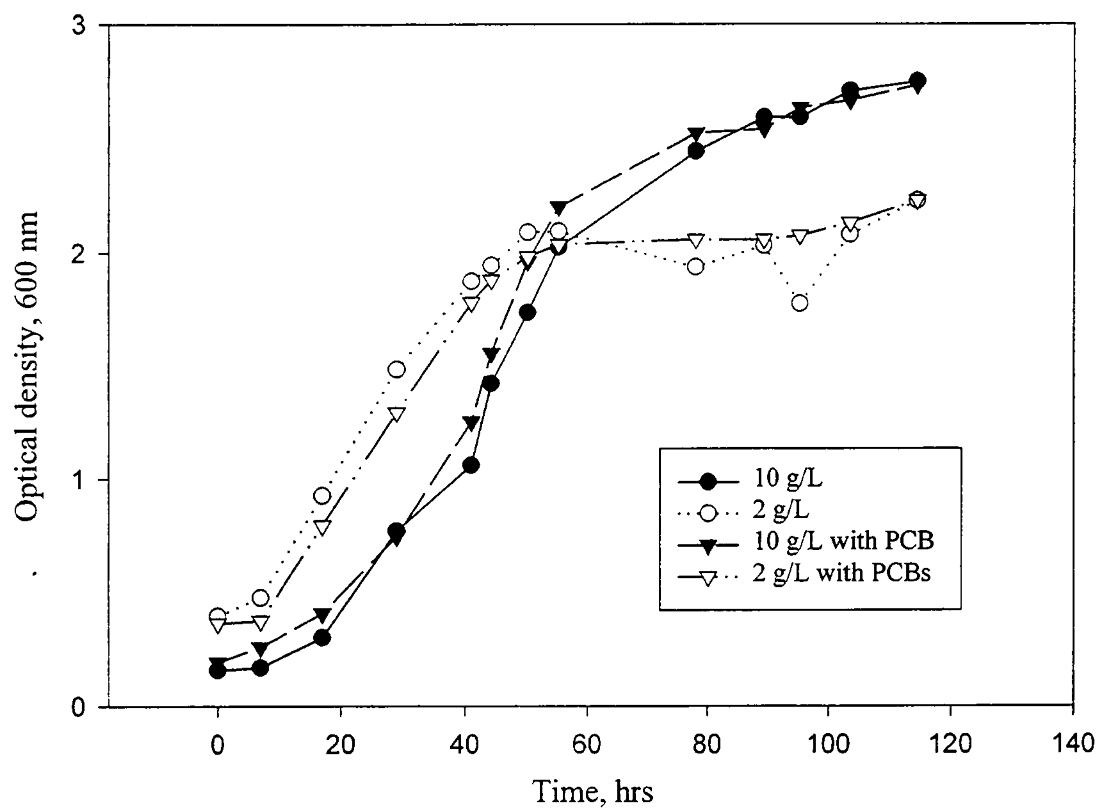


Figure 11. Optical densities of PCB-GEMs in bioreactors with varying initial POL (10) concentrations with and without 50 mg/L Aroclor 1242

Table 7. Specific substrate utilization rate for varying surfactant concentrations with and without PCBs

Pol 10 Concentration, g/L	PCB Concentration, mg/L	Specific Substrate Utilization Rate (hr^{-1})
10	0	0.239
10	50	0.222
2	0	0.098
2	50	0.101
10*	50	0.208
7*	50	0.158
4*	50	0.097
2*	50	0.062

*Lag phase is included in the analysis

X = average biomass concentration during time interval dt, g/L protein

At 50 mg/L, Aroclor 1242 had no effect either on the metabolism of POL (10) or the growth of the GEMs.

6.3.1.1 Effect of PCB Concentration on Biodegradation

Initial concentration of Aroclor 1242 dissolved in 10 g/L of POL (10) was varied from 22 mg/L to 269 mg/L in separate 7-day biodegradation experiments. After 7 days, 64% of the PCBs in 46 mg/L of Aroclor 1242 initially added was degraded compared to 52% degradation observed for an initial Aroclor 1242 concentration of 269 mg/L (**Table 8**). Optimum biodegradation of Aroclor occurred at an initial concentration of 46 mg/L. The rate of PCB biodegradation was optimized at 269 mg/L (**Figure 12**).

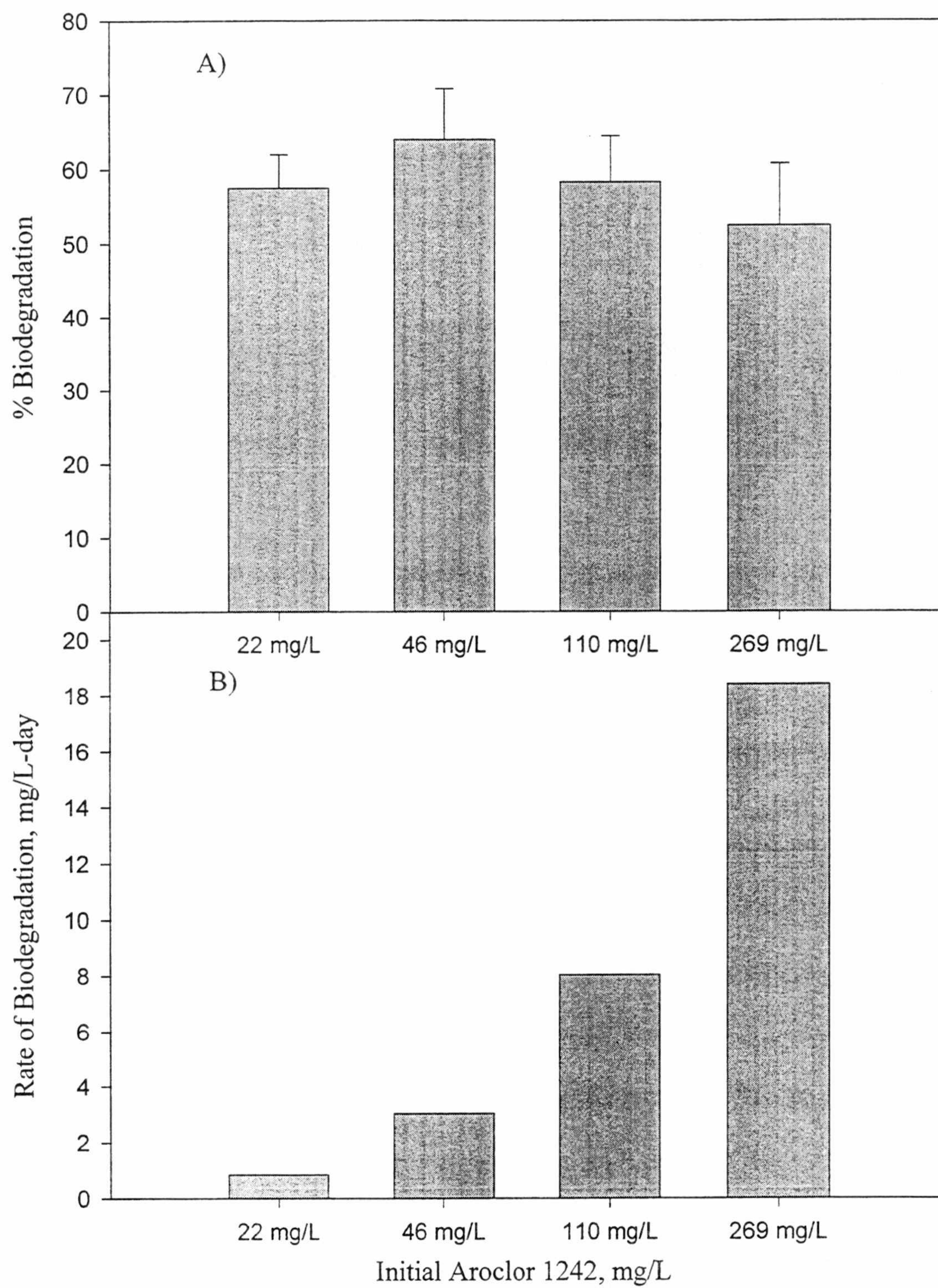


Figure 12. Effect of initial Aroclor concentration on (A) percent biodegradation and (B) rate of biodegradation

Table 8. Effect of initial Aroclor 1242 concentration on 7-day biodegradation (10 g/L of POL (10))

Initial PCB (mg/L)	**PCB Remaining (mg/L)**		**Net Degraded (mg/L)**	**Biodegraded (%)**	**Rate (mg PCBs/L-day)**
	Control	Experiment			
22	10.5	4.48	6.02	57.5	0.86
46	33.1	11.9	21.2	63.9	3.03
110	96.4	40.2	56.2	58.3	8.03
269	246	117	129	52.4	18.4

Chemostats run at the optimum initial Aroclor concentration observed above could be used to obtain valuable information on operational variables, such as throughput rate and viable GEM population.

In the concentration range studied, Aroclor 1242 seemingly did not have any toxic effect on the growth of the PCB-GEMs (**Figure 13**) or on POL (10) degradation (**Figure 14**). However, at very high concentrations of Aroclor, accumulation of known toxic metabolites, chlorocatechols, for example, is a distinct possibility. The bioreactor that initially received 269 mg/L of Aroclor, developed a gray to black color at the end of the

7-day experiment. By comparison, all other bioreactors produced a hay-yellow color, which is indicative of PCB degradation (Furukawa *et al.*, 1978). It has been documented that *Psuedomonas stutzeri* growing on biphenyl in the presence of 2-chlorbenzoate (CBA) or 3-CBA excreted black metabolites (Vrana *et al.*, 1996). These metabolites were shown to decrease biphenyl metabolism. Another study showed that the presence of mono- and dichlorobenzoates inhibited Aroclor 1242 degradation (Sylvestre, 1995).

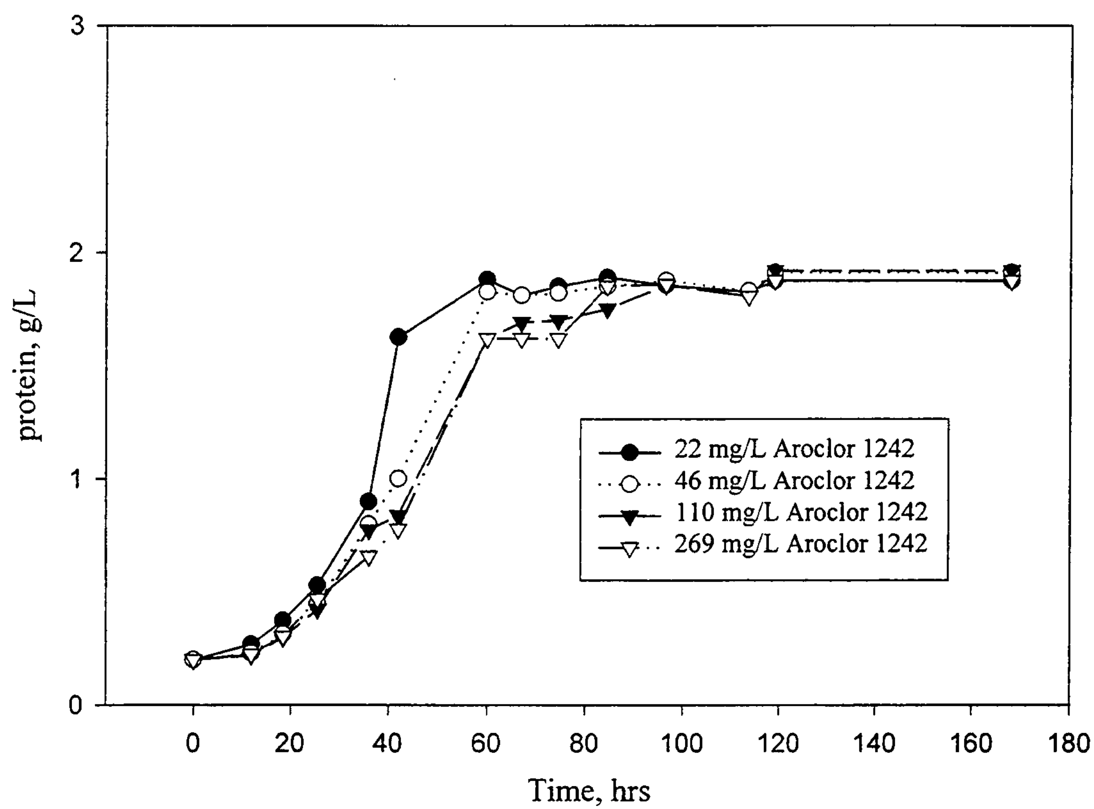


Figure 13. Growth of PCB-GEMs with varying concentrations of Aroclor 1242 at an initial POL (10) concentration of 10 g/L

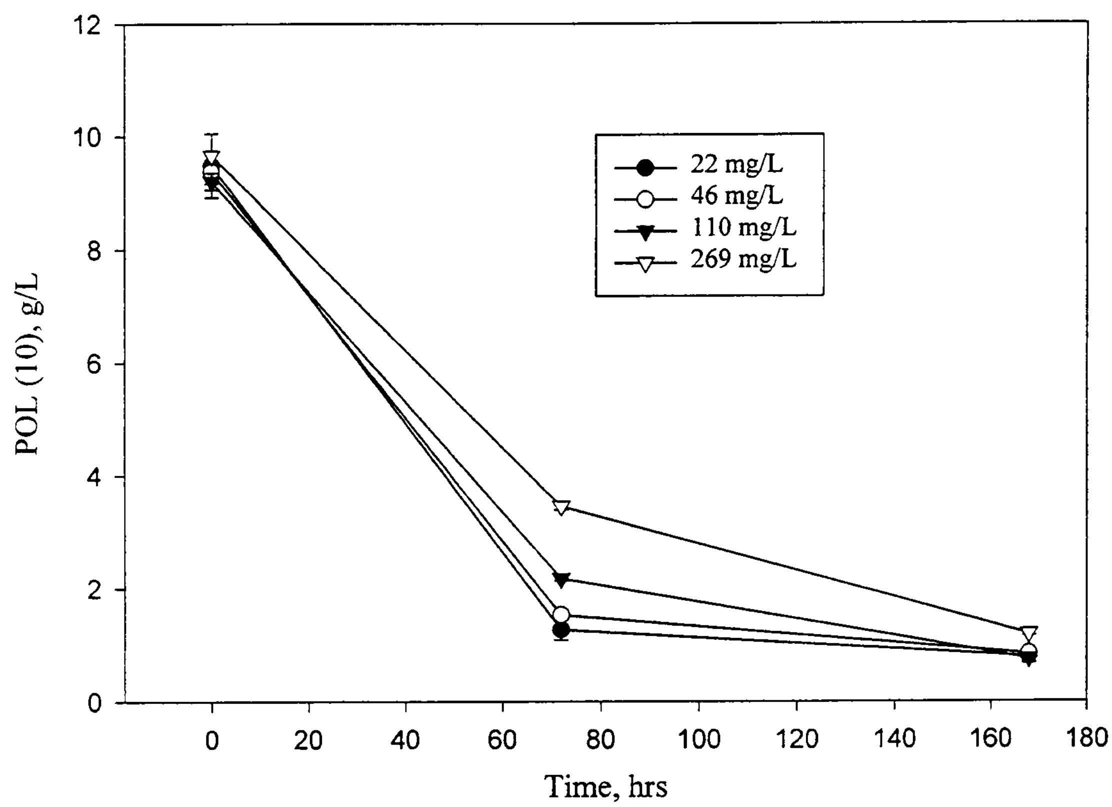


Figure 14. POL (10) biodegradation at varying PCB concentrations

Also, the strain IPL5::TnPCB when grown in the presence of benzoate produced a brown metabolite, whereas this was not seen in benzoate-grown cultures of the parent strain (IPL5) (Lajoie *et al.*, 1997). This suggested interference between the indigenous benzoate pathway and the biphenyl operon (Lajoie *et al.*, 1997). A yeast cell growth inhibition assay (**Section 5.6**) was also performed on the supernatant from the suspect bioreactor after the 7-day study. There was evidence of some toxic metabolites being present. A comparison of the growth of yeast in pure Aroclor 1242 with that in the control and experimental reactors is shown in **Figure 15** (no estrogenic activity was detected for any of these samples). Typically, chlorinated compounds, upon biodegradation, produce toxic metabolites. These metabolites did not seem to inhibit growth, which reached a maximum in two days, well before significant biodegradation of PCBs occurred. However, toxicity might have caused a decrease in enzyme activity observed after 110 hours in the suspect bioreactor, as illustrated in **Figure 16**, while the activity in all other reactors remained relatively steady from 110 to 170 hr when the experiment was terminated.

6.3.1.2 Effect of POL (10) on PCB biodegradation

It was hypothesized that high dosages of surfactant would encourage production of larger amounts of the PCB-degrading enzyme and PCB degradation would be improved. However, high POL (10) dosages failed to improve PCB biodegradation. In fact, initial surfactant concentration, 2 g/L to 10 g/L, was shown to be inversely proportional to PCB biodegradation. The growth of the GEMs, represented by whole cell protein and optical

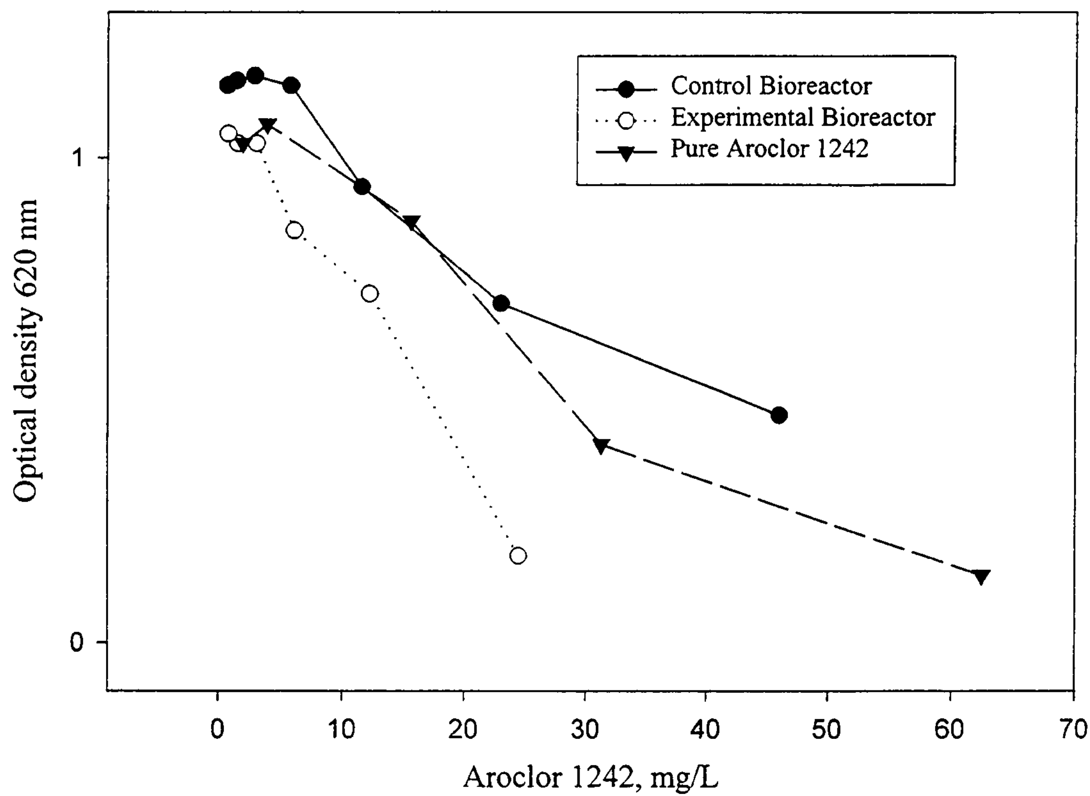


Figure 15. Inhibition of yeast cell growth by PCBs with 10 g/L initial POL (10)

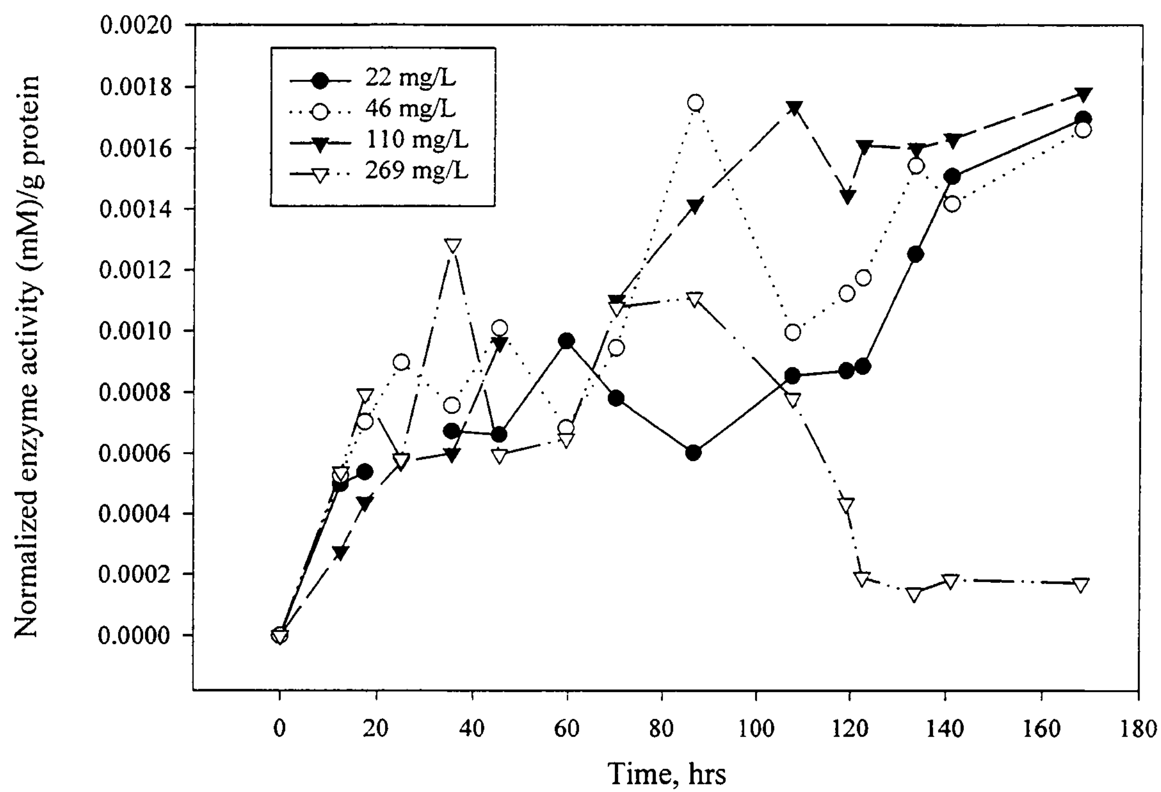


Figure 16. Normalized enzyme activity for varying Aroclor1242 concentrations

density, are shown in **Figure 17**. Both parameters increased with surfactant concentration. Also, POL (10) biodegradation is shown in **Figure 18**.

Competitive Inhibition in PCB Biodegradation: In 7-day experiments, PCB biodegradation was shown to be inversely proportional to the initial POL (10) concentration in the range of 2 g/L to 10 g/L (**Table 9**). It could not be explained by abiotic factors. In fact, the chromatograms showed that biodegradable congener peaks were preferentially attenuated when an initial concentration of 2 g/L of POL (10) was used as compared to that at a POL (10) dosage of 10 g/L (**Figure 19**). The largest destruction of PCBs occurred (74%) at a POL (10) dose of 2 g/L. The concentrations of PCB fluctuated in the control bioreactors, whereas the PCBs decreased steadily over time in the experimental bioreactors (**Figure 20**). After an extended period of biodegradation (24 days), the same percentages of PCBs were biodegraded as in 7 days. However, during this same time period, a large mass of PCBs was removed from solution. This removal was assumed to be abiotic. **Table 11** shows that after 24 days, lesser amount of PCBs were biodegraded than that after 7 days.

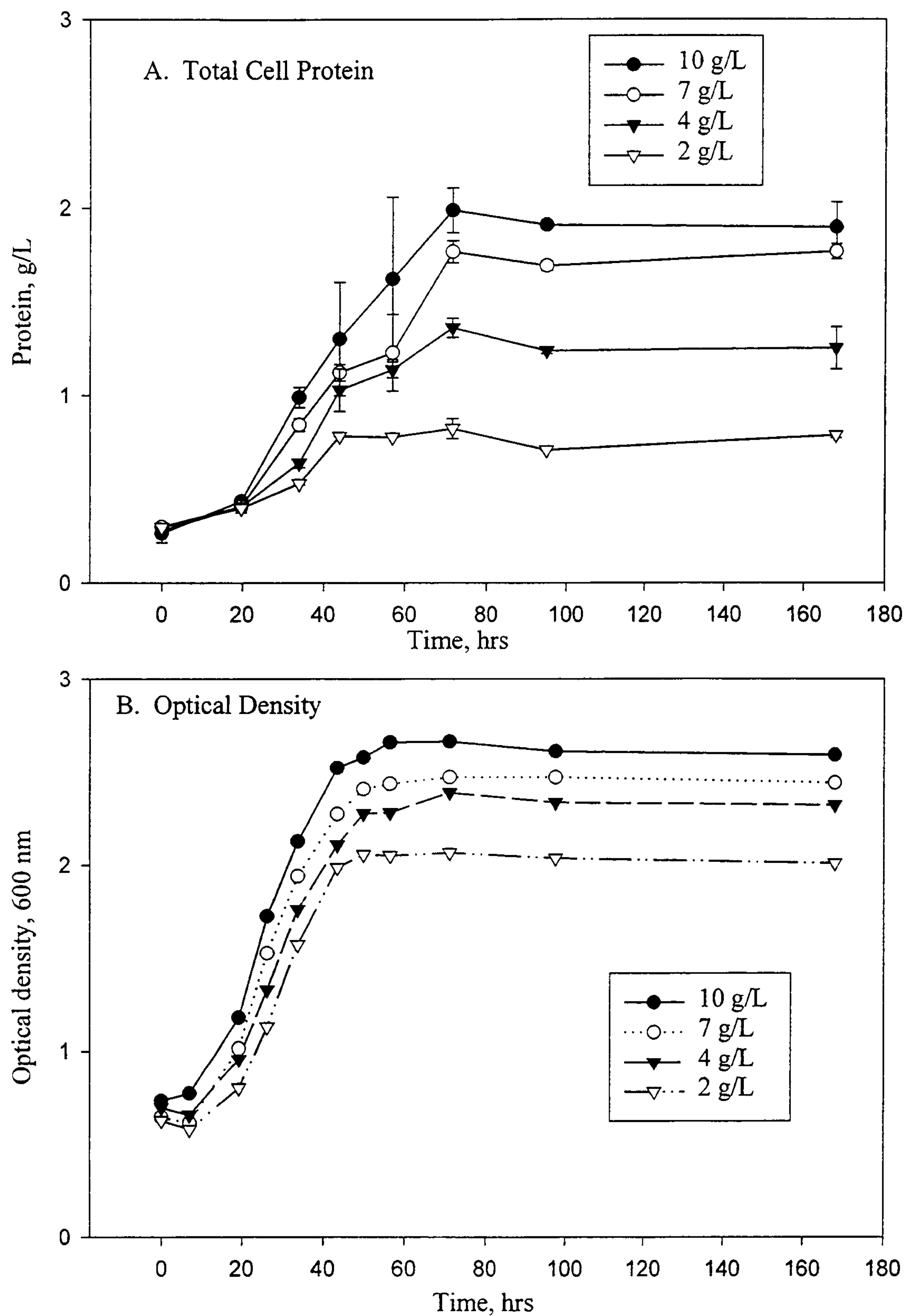


Figure 17. Growth of PCB-GEMs at varying POL (10) concentrations (A) total cell protein and (B) optical density

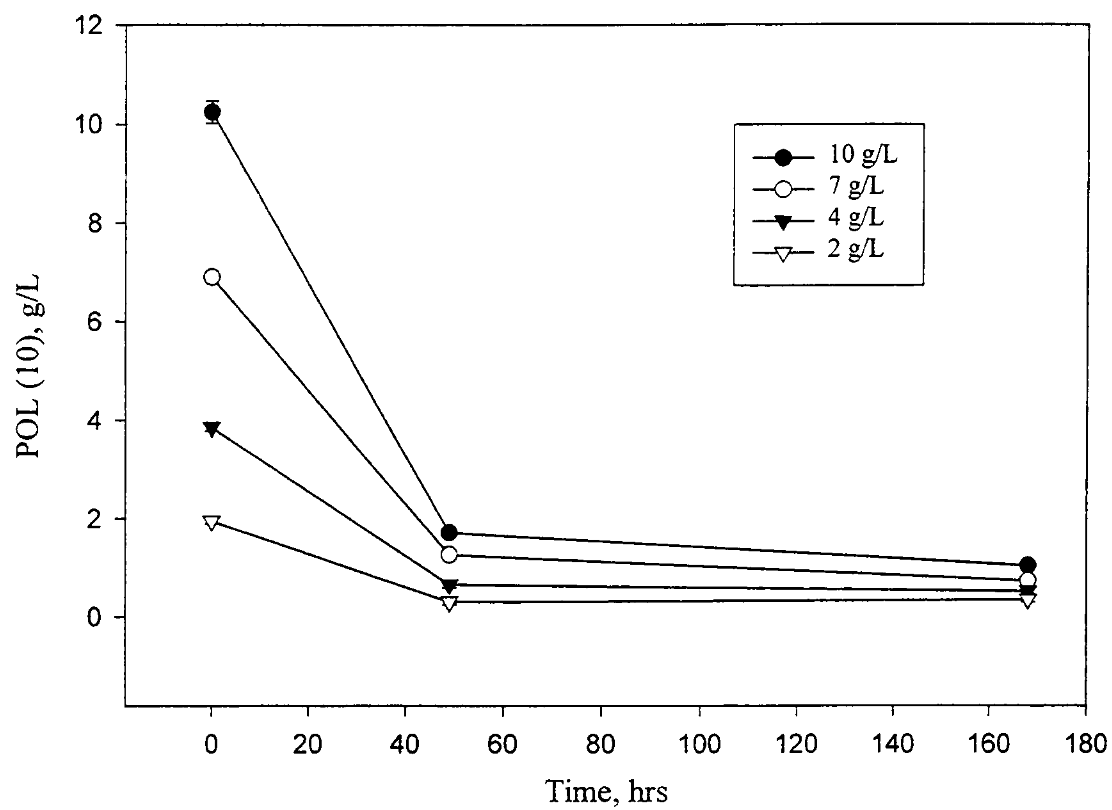


Figure 18. POL (10) biodegradation at varying POL (10) starting concentrations

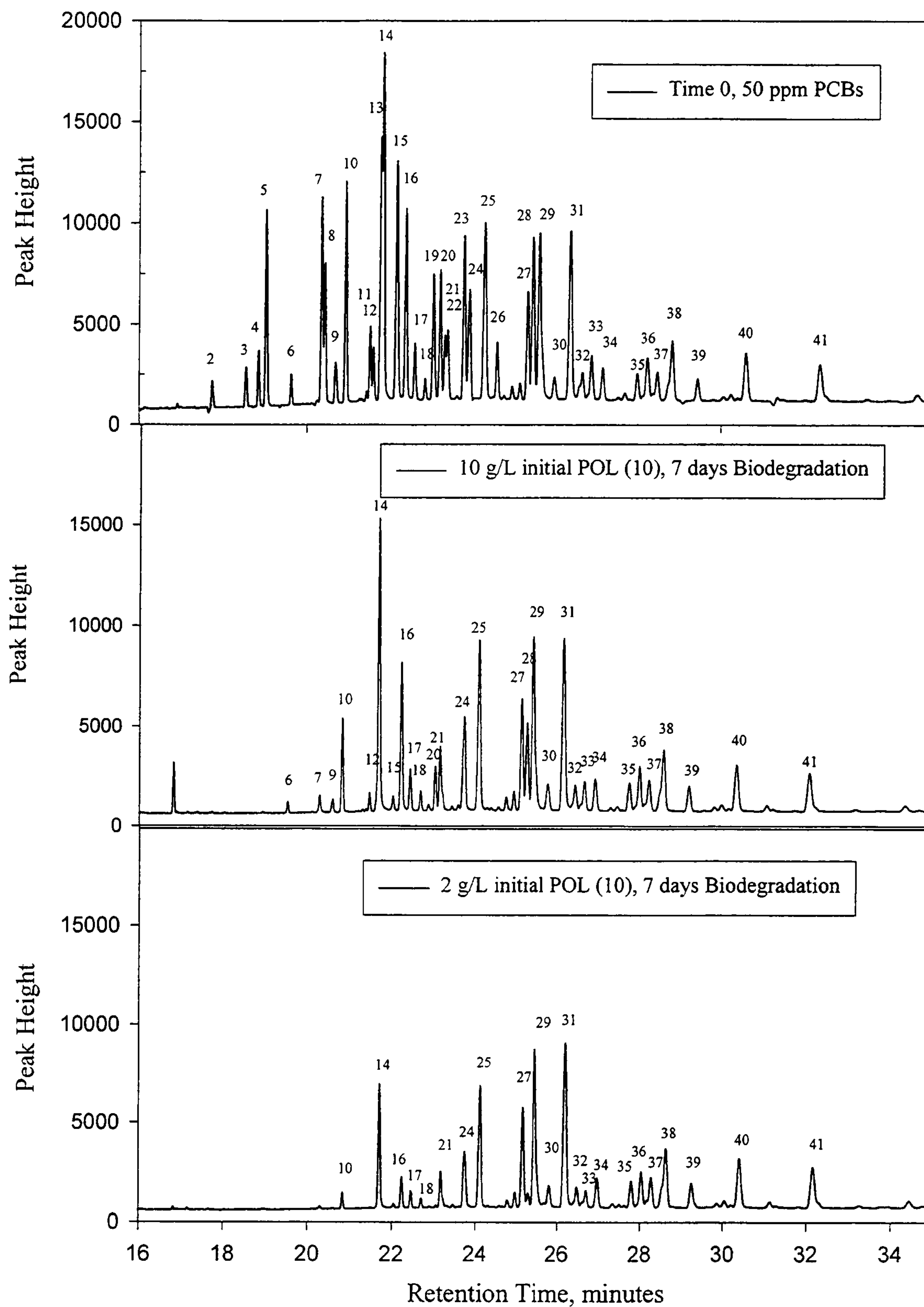
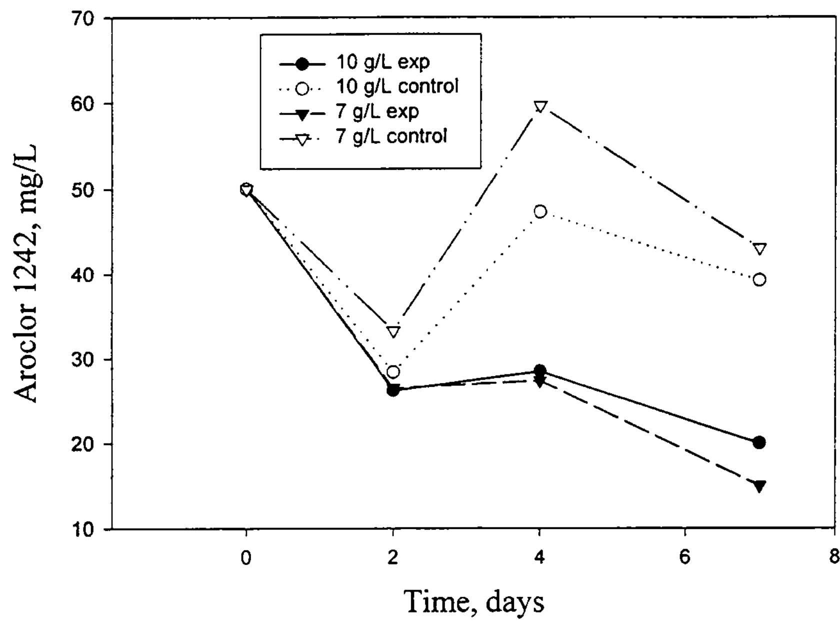


Figure 19. Chromatogram comparisons for varying surfactant concentrations



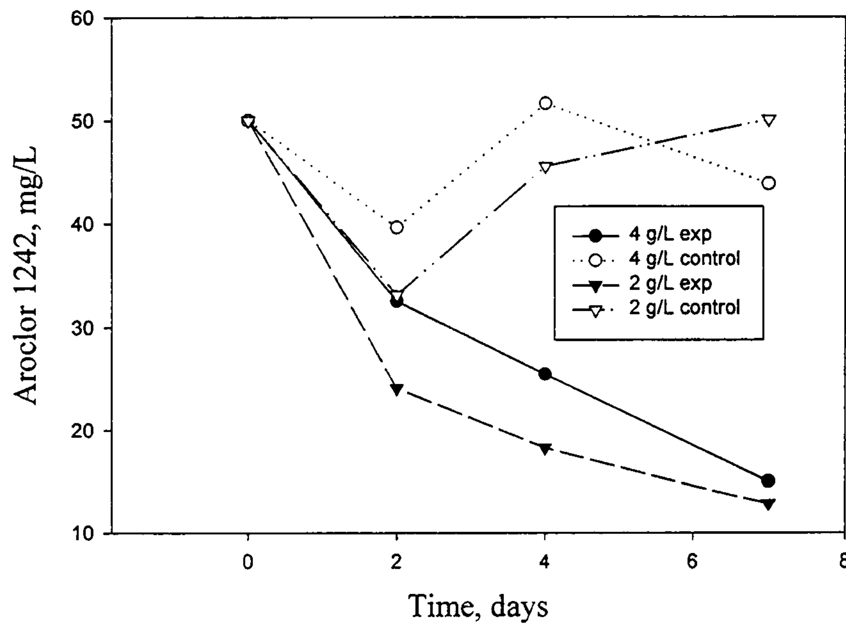


Figure 20. Aroclor 1242 biodegradation at varying surfactant concentrations (10 g/L, 7 g/L, 4 g/L, 2 g/L)

Table 9. PCB biodegraded in 7 days in the presence of 50 ppm of Aroclor 1242

Initial POL (10) g/L	Final POL (10), g/L	Control PCB, mg/L	Experi-mental PCB, mg/L	Biodegra dation (%)	Biodeg Rate after 7 days (mg/L-day)	Max Biodeg Rate (mg/L-day)
10	1.0	39.2	20	49.0	2.75	4.7[b]
7	0.7	43.1	15.0	65.2	4.01	8.08[b]
4	0.5	43.8	15.1	65.5	4.10	6.55[b]
2	0.3	50.1	12.8	74.4	5.33	6.8[b]
1[a]	0.3	11.3	4.8	57.5	0.93	15.4[c]
0.5[a]	0.2	12.6	7.9	37.3	0.67	9.2[c]

[a]These concentrations performed as a separate experiment
[b]4 days biodegradation
[c]2 days biodegradation

Table 10: Rate of PCB biodegradation (mg/L-day)

	Initial POL (10) Concentrations					
Time, days	**10 g/L**	**7 g/L**	**4 g/L**	**2 g/L**	**1 g/L***	**0.5 g/L***
0	0	0	0	0	0	0
2	1.05	3.40	3.55	4.50	7.70	4.60
4	4.7	8.08	6.55	6.8	2.1**	1.55**
7	2.75	4.01	4.10	5.33	0.93	0.67
24*	0.46	0.65	0.50	-	-	-

*These concentrations were run as a separate experiment
**Hypothesize abiotic removal

The initial POL (10) concentration of 1 g/L obtained the best biodegradation results after two days (**Table 10**). However, biodegradation ceased after two days. After two days, the internal standard (the non-biodegradable Aroclor 1242 peak #41) decreased by a factor of two. This indicated that a sufficient concentration of POL (10) was not present in solution after two days, and **Figure 21** shows a steady decrease of PCBs in the control bioreactors. A comparison of percent biodegradation and maximum rate of biodegradation is shown in **Figure 22**. Also, chemostat experiments run at 1 g/L POL (10) may perform much better than higher concentrations of surfactant.

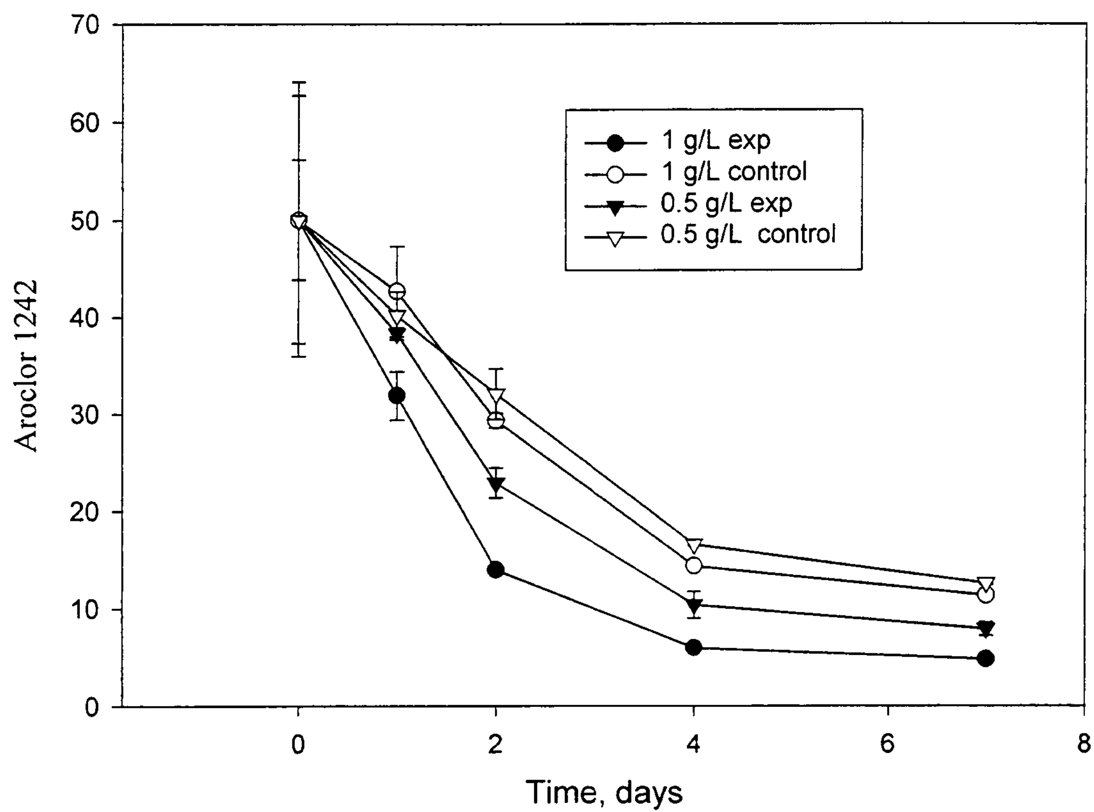


Figure 21. Biodegradation of Aroclor 1242 at varying POL (10) concentrations (1 g/L, 0.5 g/L)

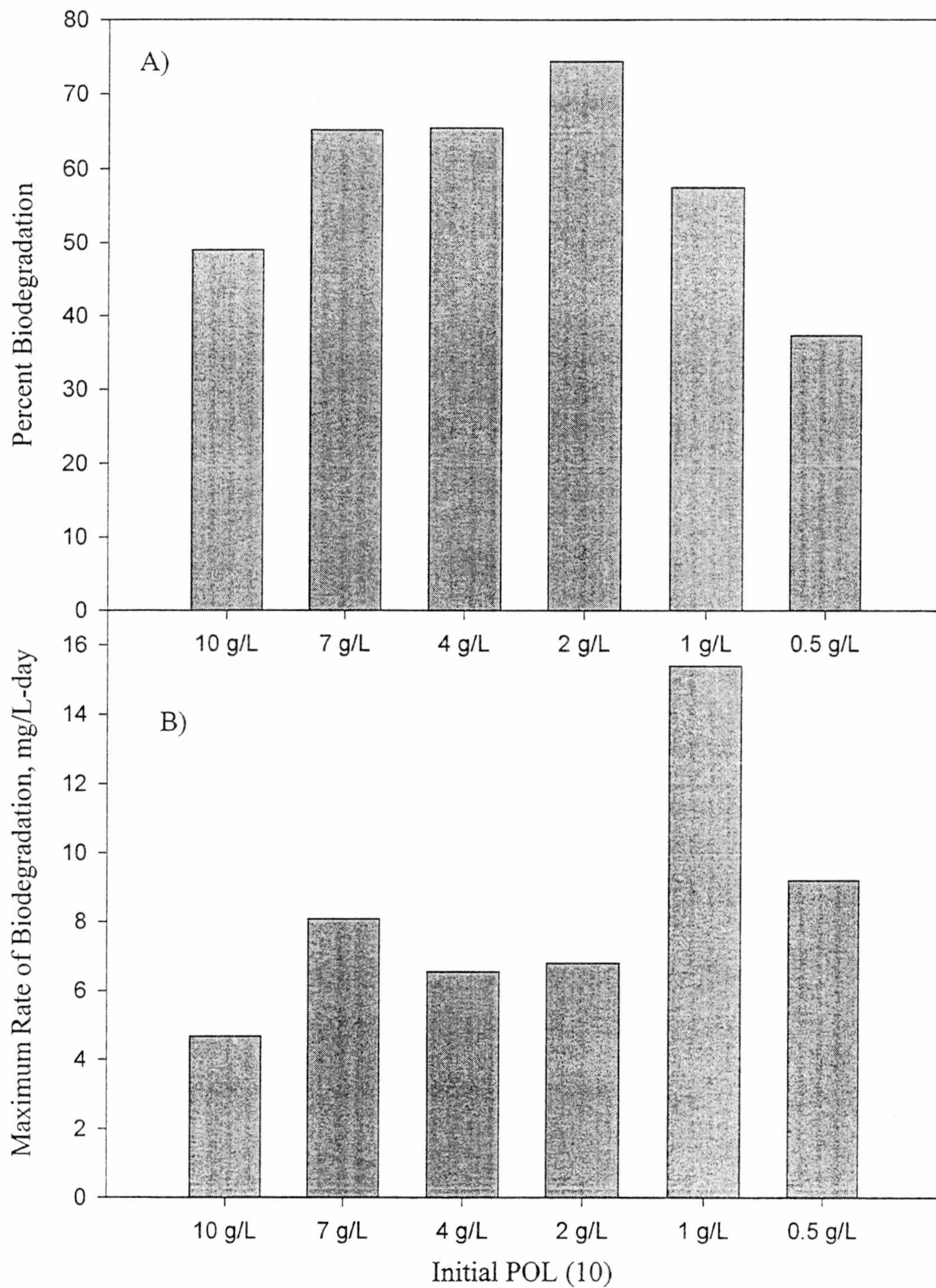


Figure 22. (A) Percent biodegradation and (B) maximum rate of biodegradation (mg/L-day) for varying initial POL (10) concentrations

As noted earlier (**Section 4.4.1**), the activity of the *bphA-D* enzyme system depends on the availability of O_2. A PCB congener molecule must simultaneously combine with *bphA* and molecular oxygen to form 2,3-dihydroxy-4-phenylhex-2,4-diene in the first step of biodegradation. This transformed PCB molecule does not register in the chromatogram as one of the original 41 peaks of Aroclor 1242. Hence, if any of the above mentioned three reactants (*bphA*, PCBs, O_2) are not in sufficient supply, PCB biodegradation may not occur.

Unfortunately, there is no viable technique to measure the enzyme directly. Thus, the *bphC* enzyme, which could be measured, was assumed to represent the level of *bphA* activity within the cell. As expected, the maximum normalized enzyme activity (*bphC*) was proportional to the initial surfactant concentration (**Figure 22**). However, the amounts of as *bphA* may not be the same. *bphC* is downstream from *bphA* on the gene; transcription could encode for but cease before reaching *bphC*. Thus, measurement of *bphC* alone could conceivably underestimate *bphA* activity. However, there should not be a large difference between the amounts of *bphA* and *bphC* produced since these two genes are on a single operon with one promoter. Further, half-life of *bphA* could be somewhat less than that of *bphC*. Though biodegradation essentially ceased by the seventh day, the activity of *bphC* continued unabated well beyond that. Also, enzyme specificity of *bphA* and *bphC* may be different. The measurement of enzyme activity

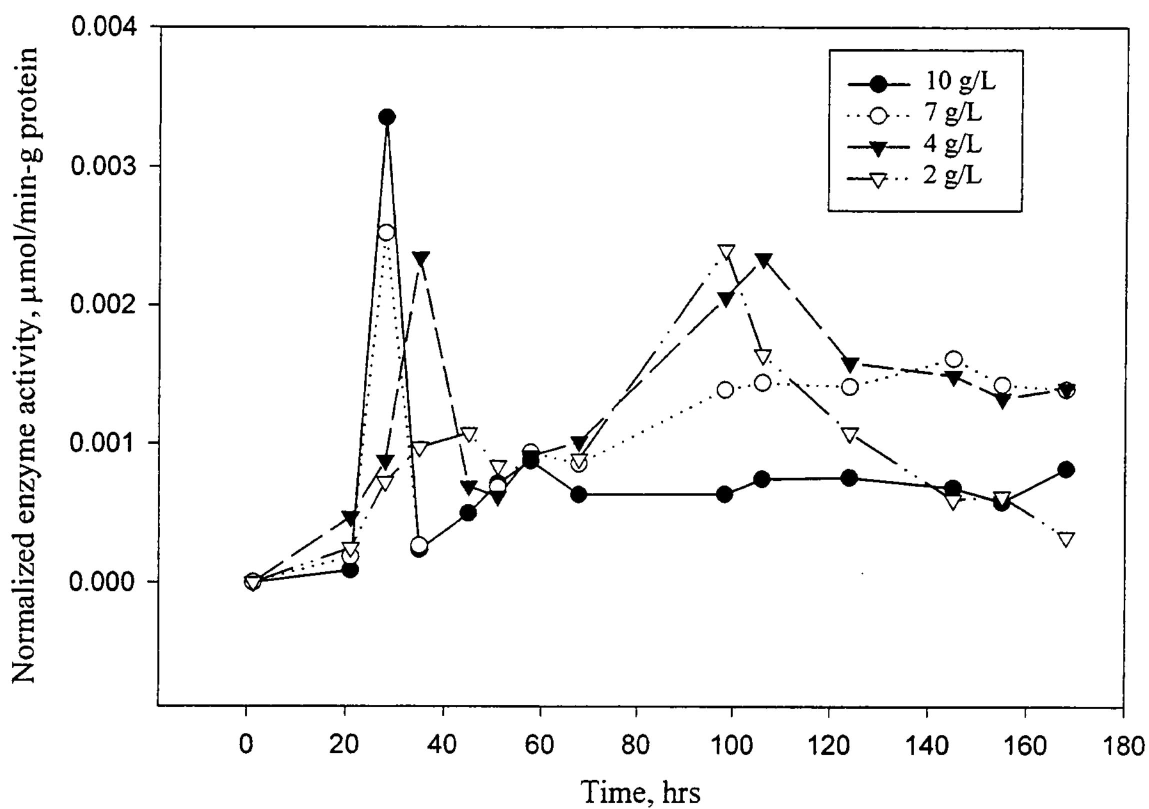


Figure 23. Normalized enzyme (*bphC*) activity (μmol/min-g protein) versus time for varying initial POL (10) concentrations

uses a substance (2,3 dihydroxybiphenyl) with a high affinity for the 2,3 dihydroxygenase enzyme.

Though seemingly sufficient O_2 oxygen was present in the reactor to support respiration of the two GEMs, enough O_2 may not have been there to support the chemical reaction between the PCB congener, *bphA*, and O_2. Competition for O_2 between respiration and activity of an O_2-dependent enzyme has also been demonstrated for the *lux* enzyme which produces bioluminescence (Applegate, *et al.*, 1998). This competition was also demonstrated by batch cultures of 2,4-D degrading bacteria (Shaler and Klecka, 1986). Also, many dioxygenase enzymes have affinities for oxygen (K_{do}) much higher than the accepted critical dissolved oxygen concentration for aerobic bacteria (**Table 4, Section 4.4**).

To test unavailability of O_2 for enzyme reaction, additional experiments were conducted where dissolved oxygen (DO) and biological growth (OD) in the reactor were carefully monitored and intrinsic oxygen uptake rate of the GEMs (**Figure 24**) were separately measured. At no time did DO fall below 5 mg/L; rarely it was less than 6 mg/L (**Figure 25a**). Cell growth (**Figure 25b**) corresponded well to DO depletion. Cell growth in the bioreactor with 2 g/L surfactant increased quickly attaining a maximum in about 60 hrs. Correspondingly, DO decreased rapidly, but returned to its original concentration in less than forty hours. On the other hand, the cells in the bioreactor that receive 10 g/L POL (10) took three days to reach maximum growth and DO returned to its original concentration in seven days after reaching its lowest level in one day. However, a DO

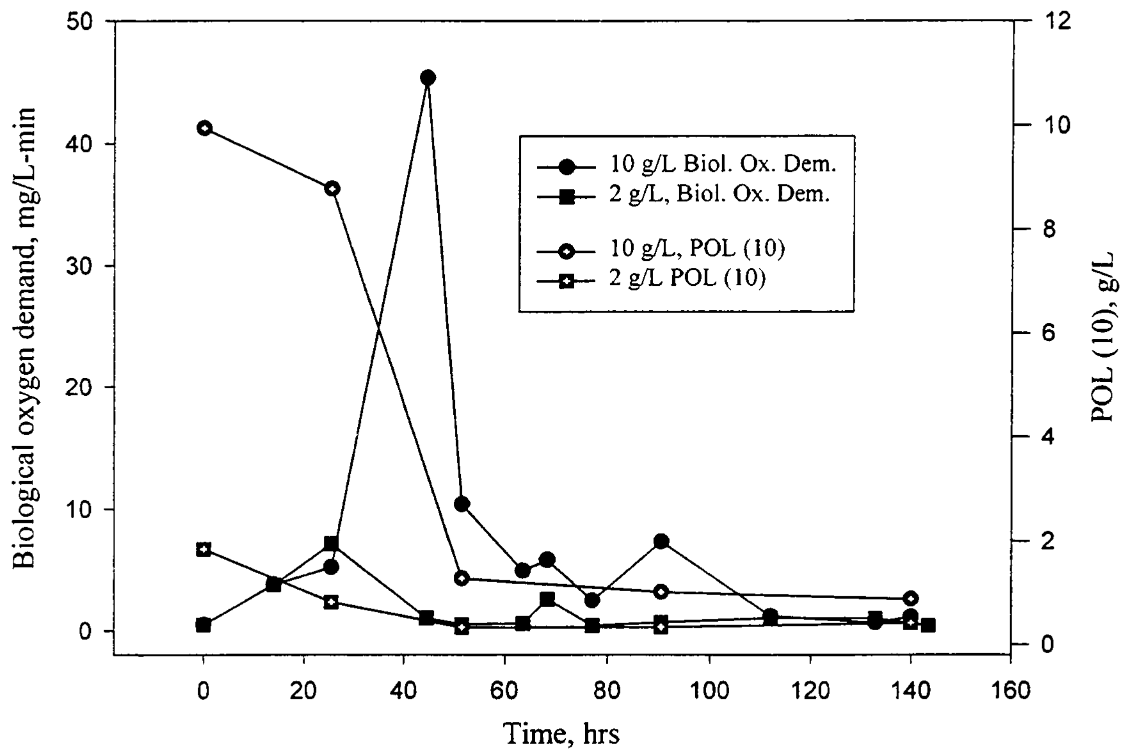


Figure 24. Biological oxygen demand and POL (10) degradation for initial POL (10) concentrations of 2 g/L and 10 g/L

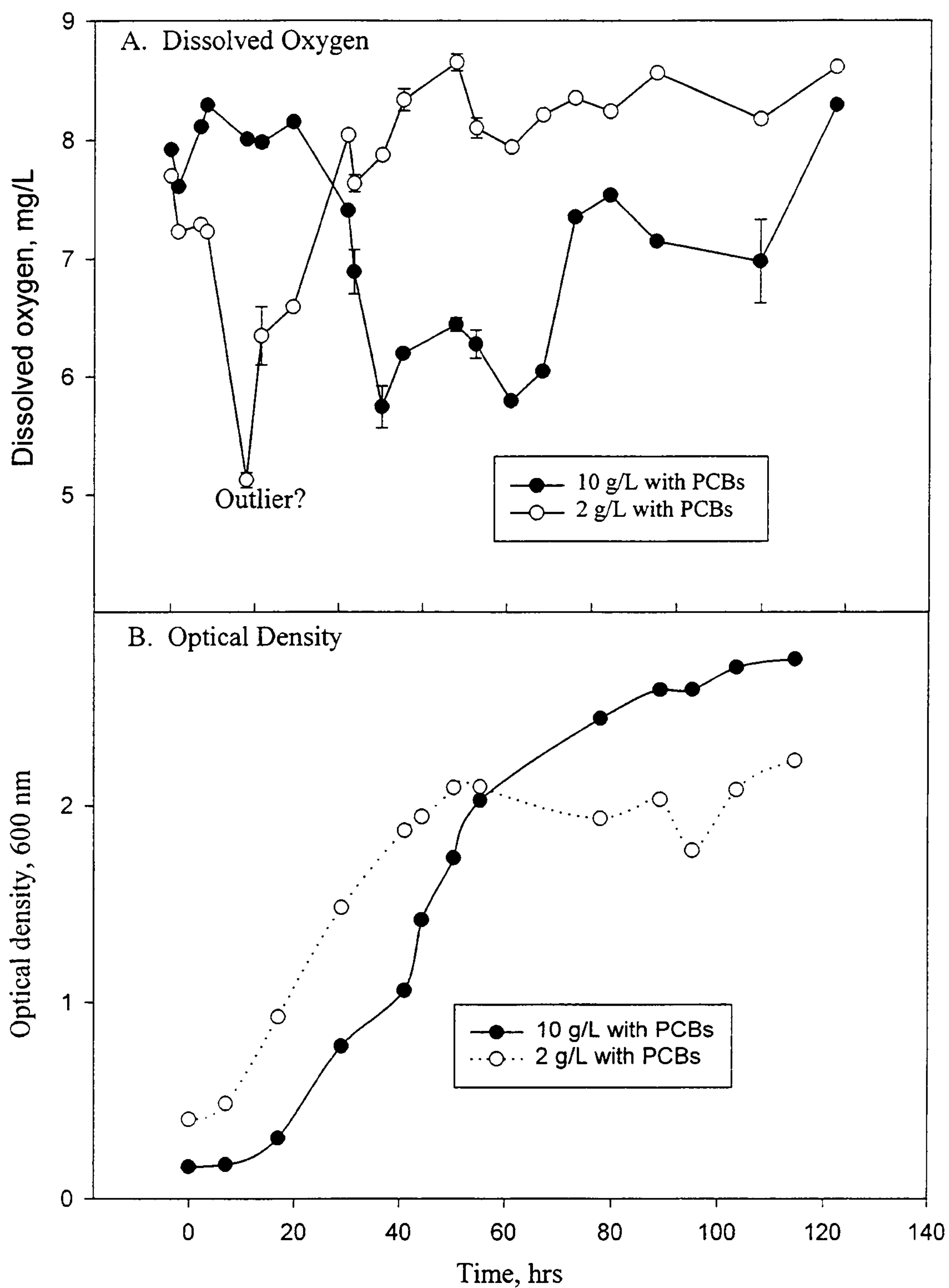


Figure 25. A) Dissolved oxygen concentration (top) and (B) optical density (bottom) for varying initial POL (10) concentrations

above 5 mg/L was always maintained in the reactors, sufficient to support aerobic cell growth.

A respirometer was used to measure intrinsic O_2 uptake rate at various times during the biodegradation experiment (See **Section 4.11** for methodology). The results are shown in **Figure 24**. In a 25.5 hr period the bacteria in the 2 g/L bioreactor degraded 1.52 g/L of surfactant. This corresponded to an oxygen demand of 1.09 mg/L-min (See Appendix, **Section 8.1** for calculations). In a 26 hr time period, the bacteria in the 10 g/L bioreactor degraded 8.66 g/L of surfactant, which corresponded to an oxygen demand of 6.10 mg/L-min. The measured peak oxygen demand (by respirometry) was 7.0 mg/L-min and 45.2 mg/L-min for 2 g/L and 10 g/L bioreactors, respectively. This data showed that the oxygen demand by the bacteria was even higher than the theoretical. Observed oxygen demand is probably higher than the experimental oxygen demand because oxygen is used for more reactions than just surfactant degradation.

6.3.2 Co-metabolism of PCBs in Photolyzed Surfactant Solutions

Maximum percent biodegradation of Aroclor 1242 over a seven day incubation was found to work well in the concentration range of 50 to 110 mg/L and at POL (10) concentrations less than 10 g/L (**Tables 8 and 9**). However, highly chlorinated congeners were not degraded. However, UV-irradiation at 254 nm has been used to successfully dechloriante these (Shi *et al.*, 1998). A suite of biodegradation experiments

was conducted to determine if photolysis contributed toward improved biodegradation without producing any acutely toxic by-products.

Section 6.2 describes the results of photolysis. All experiments on photolysis in this part of the study was conducted at a POL (10) concentration of 4 g/L. Two concentrations of photolyzed Aroclor 1242, namely 78 mg/L and 210 mg/L, were used in separate biodegradation experiments with attendant control reactors. Following the addition of nutrients and bacterial inocula, the actual concentration of POL (10) was 3.2 g/L and that of PCB was 56 mg/L and 154 mg/L in the two reactors.

After six days, no evidence of acute toxicity due to the photolysis by-products could be detected. Growth, as measured by protein, and surfactant degradation were nearly identical in photolyzed and unphotolyzed PCB solutions (**Figure 26**). The specific substrate utilization rate of POL (10) in the photolyzed and unphotolyzed experimental solutions corresponded closely (0.140 hr^{-1}, 0.132 hr^{-1}, respectively). Further, *bphC* activity was nearly equal in the two solutions (**Figure 27**). Seemingly, photolysis of Aroclor 1242 did not create by-product acutely toxic to the PCB-GEMs. The PCB removal by the three treatment modes (photolysis alone, biodegradation alone, and the combination of photolysis and biodegradation) are compared in **Table 11**. Also, PCB removal is shown in **Figure 28**.

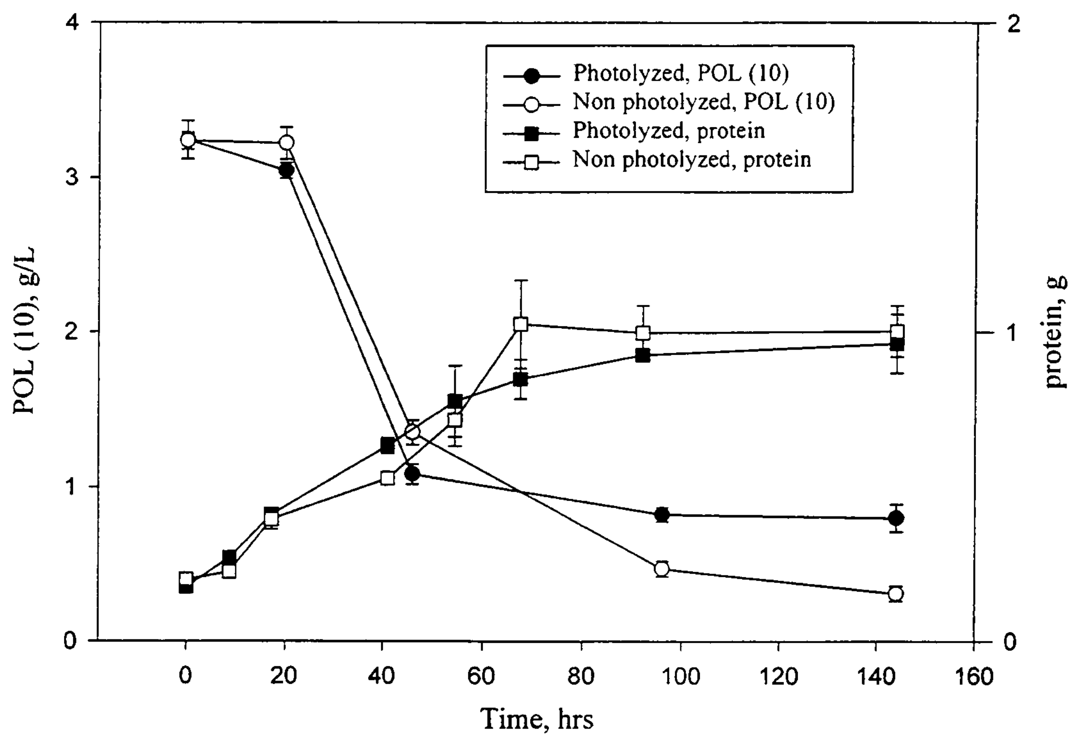


Figure 26. Comparison of surfactant biodegradation and bacterial growth in photolyzed and non photolyzed samples

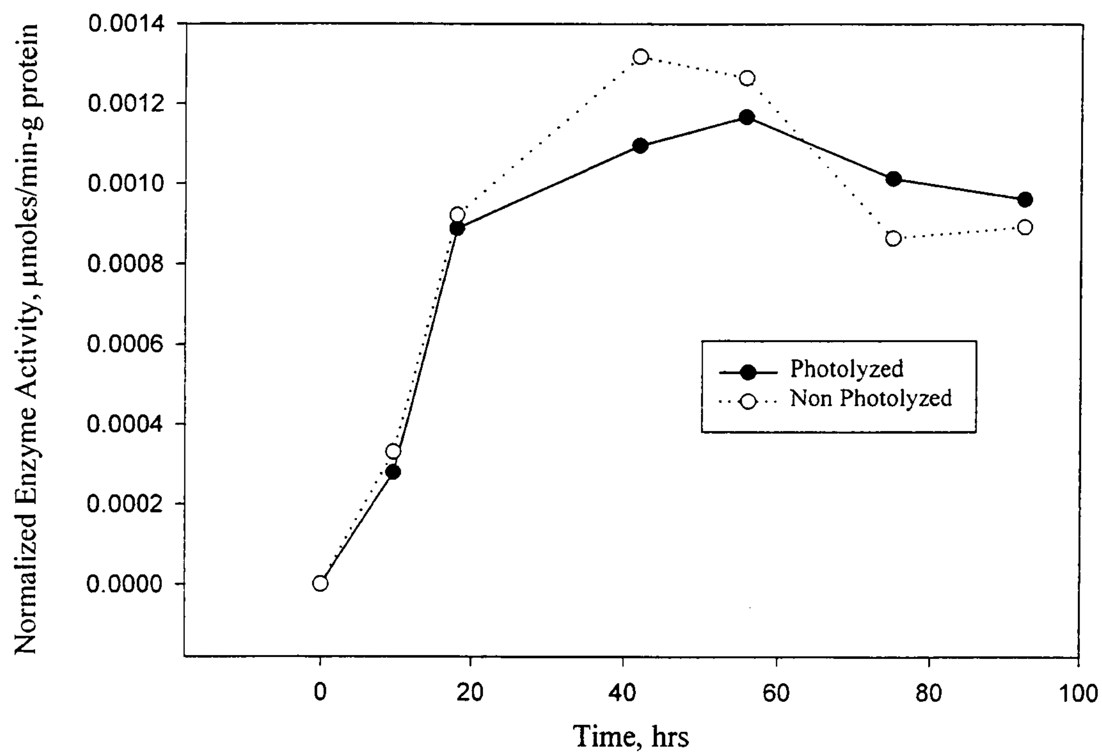


Figure 27. Normalized enzyme activity in photolyzed and non photolyzed Aroclor 1242 solutions

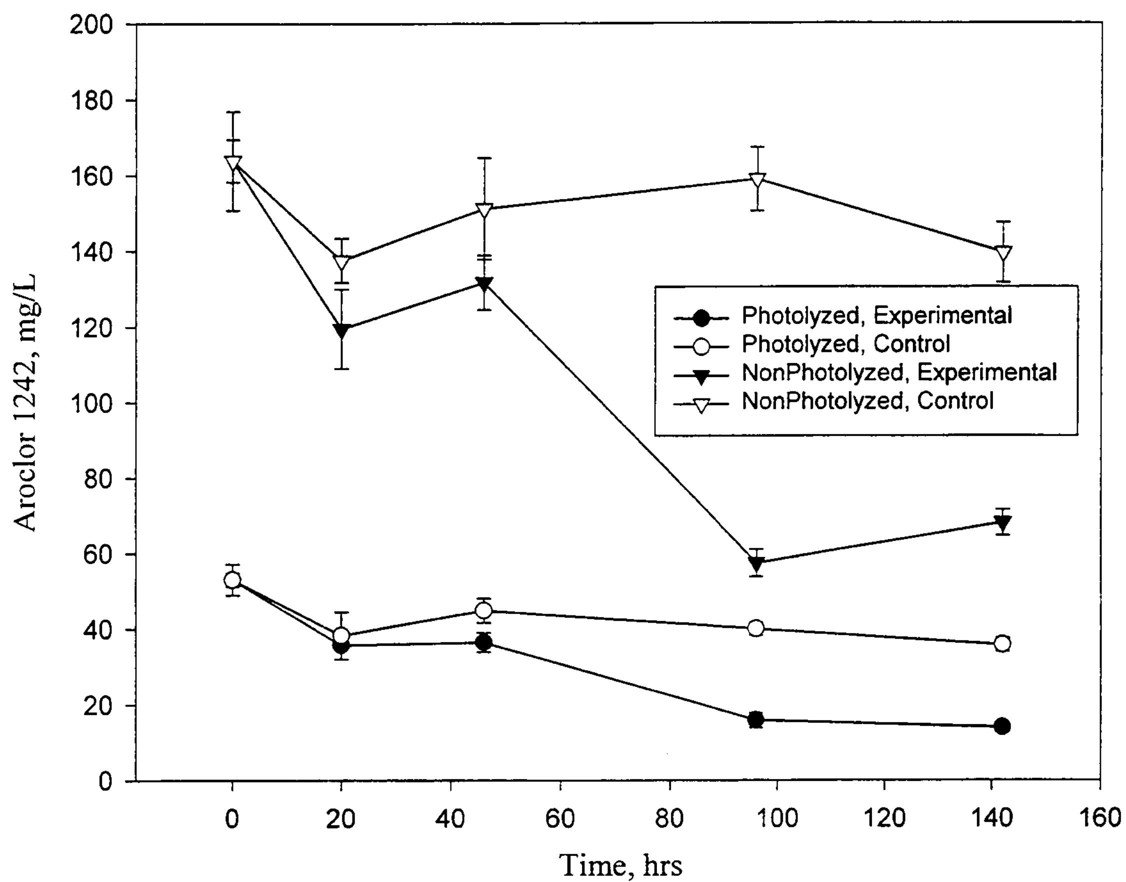


Figure 28. Biodegradation of photolyzed and non photolyzed Aroclor 1242 solutions

Table 11. Biodegradation of PCBs by different treatment methods

Treatment	Percent of PCBs Removed
Photolysis only (40 min)	63.0
Biodegradation only (6 days)	51.6
Biodegradation (6 days) after Photolysis (40 min)	60.1
Overall Removal, Biodegradation (6 days) and Photolysis (40 min)	85.2

Overall, combination of photolysis and biodegradation removed more PCBs than by either method alone (**Figure 29** and **Figure 30**). Conceivably, prolonged photolysis alone could destroy nearly all congeners in the PCB mixture as illustrated in by **Figure 9**. However, such a treatment regime would be prohibitively expensive. The comparison of removal by photolysis and biodegradation based on percent degradation may not be entirely appropriate since no control was used in photolysis experiments. Hence, abiotic losses were ignored in calculating percent degradation using the following expression:

$$\text{Percent Removal} = [1-(C_{final}\ (\text{photolyzed})\ /C_o)]*\ 100$$

Photolysis increased removal by subsequent biodegradation, 60% in 6 days, compared to 52% removal in the same time period in the unphotolyzed solution (**Table 11**). It should be noted that only the original 41 peaks of Aroclor 1242 were used in calculating removal by photolysis although three new peaks were detected. Therefore, if biphenyl related compounds were formed by photolysis, the analysis did not account for them. Since *bphC* activity was identical in photolyzed and unphotolyzed solutions, presumably these new PCB derivatives were also being degraded. Photolysis of pure 2,2',4,4' PCB

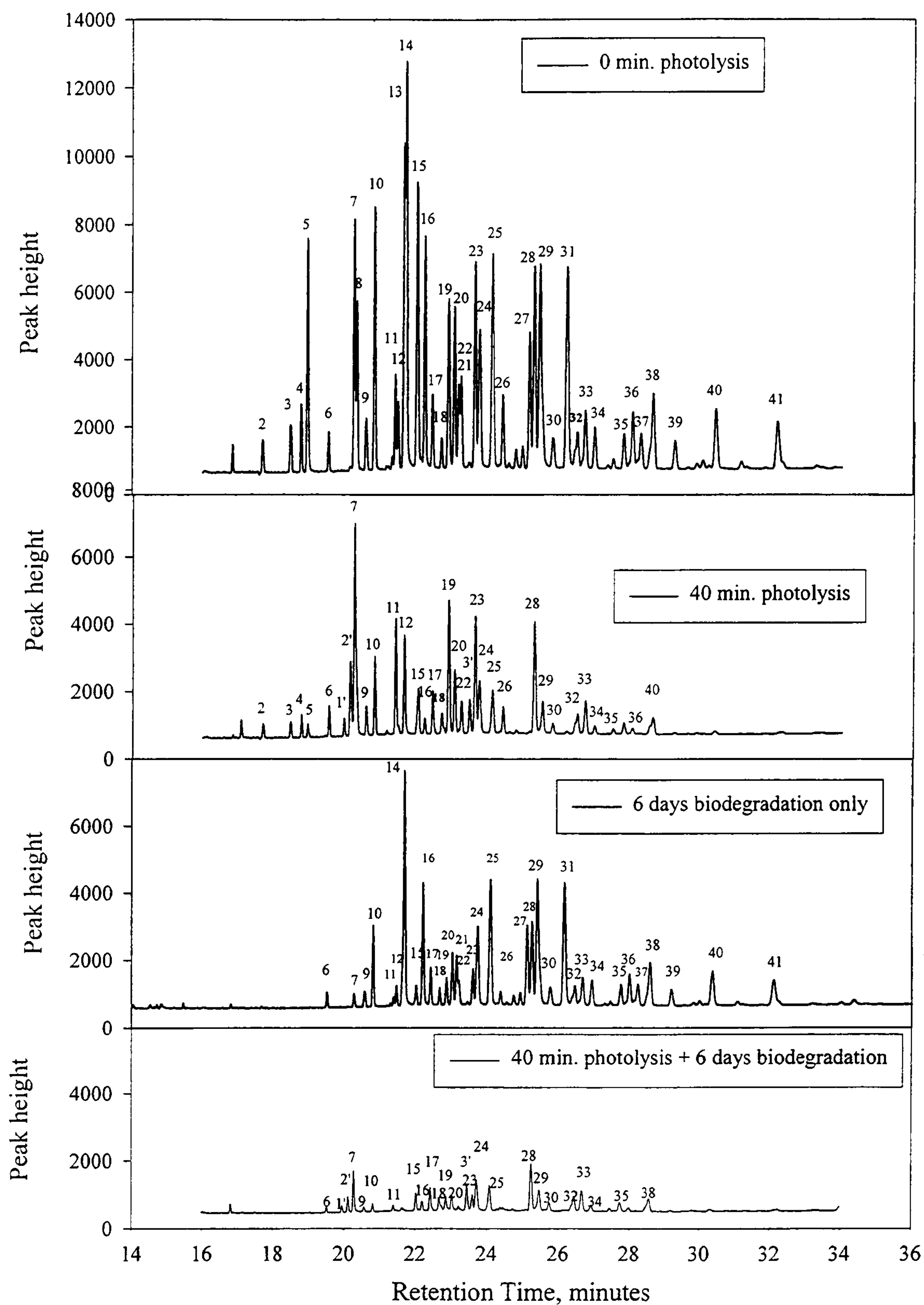


Figure 29. Chromatograms of Aroclor 1242 in POL (10) solution at 0 minutes photolysis, 40 minutes photolysis, 6 days of biodegradation, and 40 minutes of photolysis prior to 6 days of biodegradation

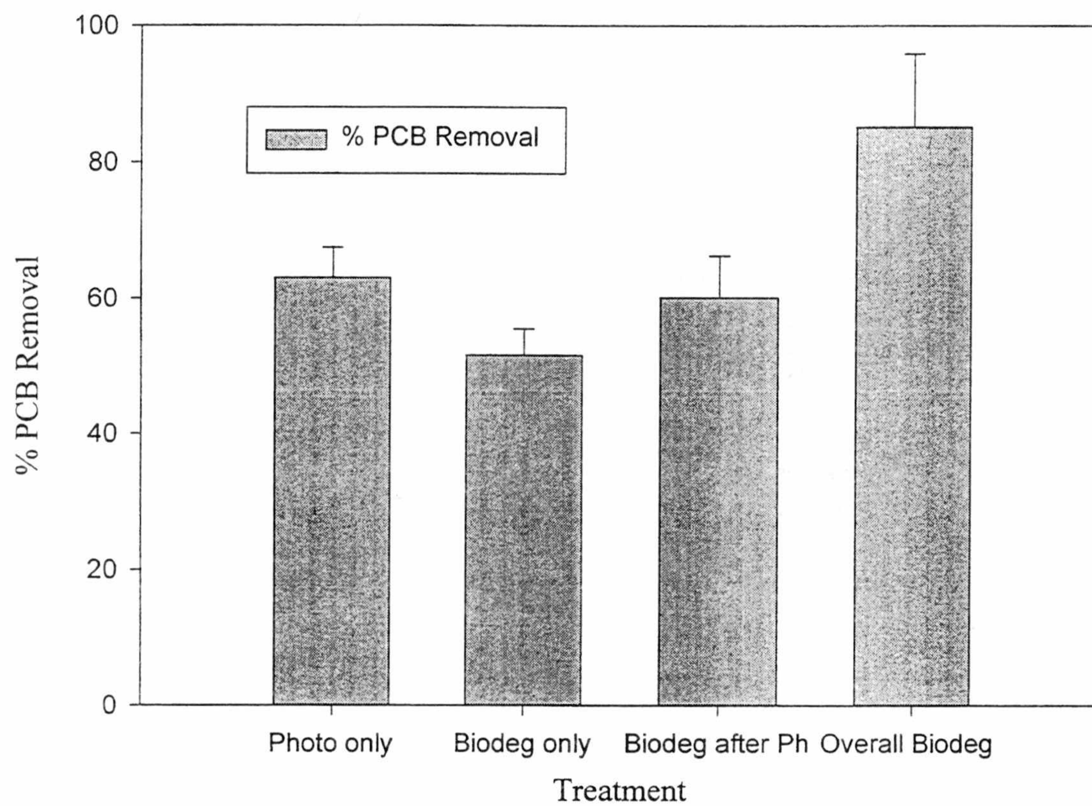
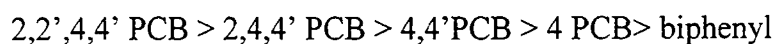


Figure 30. Comparison of PCB removal for the different treatments

revealed reductive dechlorination to be the mechanism of degrdation, shown below (Shi et. al., 1998):



Only traces of 4-PCB or biphenyl were present in the photolyzed solution whereas the concentration of 4,4'PCB was significant. Apparently, the rate of photodegradation of 4-PCB and biphenyl was much faster than that of 4,4'-PCB. Therefore, it is possible that no biphenyl was present in the follow-up biodegradation experiments. Without a complete mass balance of photoproducts, it is impossible to predict the various PCB derivatives that *bphC* is capable of degrading.

Photolysis effectively degraded the highly chlorinated congeners in Aroclor 1242. **Figure 9** clearly demonstrates the progressive attenuation of the peaks for these congeners. The resulting lightly chlorinated congeners are amenable to biodegradation. Thus, by using photolysis preceding to biodegradation, more PCBs can be removed than by biodegradation alone.

7. CONCLUSIONS

A feasible method of remediating PCBs would benefit society and the environment. An integrated treatment scheme was investigated for remediating PCB-contaminated soils. It consisted of

(1) PCB solubilization by washing soils with a nonionic surfactant;

(2) dechlorination of highly chlorinated congeners in surfactant wash water by photolysis and make them more amenable to biodegradation; and finally,

(3) biodegradation of photolyzed congeners using genetically engineered aerobic microorganisms (GEMs), capable of using surfactants for growth while cometabolizing PCBs.

At supra-CMC concentrations, POL (10) significantly increases the solubility of Aroclor 1242 (MSR = 0.6165). Photolysis was shown to degrade Aroclor 1242 in a POL (10) solution (at an initial rate constant of 5.8×10^{-4} sec^{-1}). Many of the congeners degraded were highly chlorinated and biorecalcitrant. At initial PCB concentrations varying between 22 mg/L to 269 mg/L, 52% to 64% of the PCBs were degraded in 7 days (optimum ≅ 50 mg/L), and the 269 mg/L sample gave the highest rate of biodegradation (18.4 mg/L-day). At initial POL (10) concentrations between 0.5 g/L and 10 g/L, 37% to 74% of the PCBs were degraded in 7 days (2g/L > 4 g/L > 7 g/L > 1 g/L > 10 g/L > 0.5 g/L), and 1 g/L gave the highest rate of biodegradation (15.4 mg/L-day). With UV irradiation for 40 min, 63% of the PCBs in a 210 mg/L solution could be degraded, and an additional degradation of 60% of the remainder was realized in a subsequent

biodegradation step. By comparison, only 52% of PCBs could be aerobically biodegraded without photolysis. Photolysis had no effect on bacterial growth indicating that photolysis by-products were not acutely toxic to the PCB-GEMs.

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APPPENDIX

APPENDIX

Oxygen Demand Calculations:

POL (10) = $C_{32}H_{66}O_{11}$; Molecular Weight = 626 g/mol

Formula:

$1/z(C_aH_bO_cN_d) + (2a\text{-}C)/z(H_2O) = a/z(CO_2) + (d/z)NH_3 + H^+ + e^-$

$z = 4a + b + 2c - 3d$	$a = 32$
	$b = 66$
	$c = 11$
	$d = 0$
	$z = 172$

$(2a\text{-}C)/z = 53/172$
$a/z = 32/172$

Assumptions:
1. Ammonia is the N source (NH_3CL used)
2. Fraction of organic matter oxidized for energy (fe) = 0.5
3. Fraction of organic matter converted to biomass (fs) = 0.5

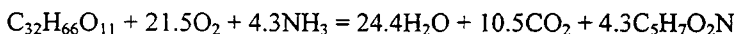
1. $1/172(C_{32}H_{66}O_{11}) + 53/172(H_20) = 32/172(CO_2) + H^+ + e^-$
2. $[1/4(O_2) + H^+ + e^- = 1/2\ H_20]fe$
3. $[1/4(CO_2) + 1/20(NH_3) + H^+ + e^- = 1/20(C_5H_7O_2N) + 2/5(H_2O)]fs$

1a. $1/172(C_{32}H_{66}O_{11}) + 53/172(H_20) = 32/172\ CO_2 + H^+ + e^-$
2a. $1/8(O_2) + 1/2H^+ + 1/2e^- = 1/4(H_20)$
3a. $1/8(CO_2) + 1/40(NH_3) + 1/2H^+ + 1/2e^- = 1/40(C_5H_7O_2N) + 1/5(H_2O)$

Add equations 1a, 2a, 3a.

$1/172(C_{32}H_{66}O_{11}) + 1/8(O_2) + 1/40(NH_3) = 24.4/172(H_20) + 10.5/172(CO_2) + 1/40(C_5H_7O_2N)$

For each mole of POL (10)


$C_{32}H_{66}O_{11} + 21.5O_2 + 4.3NH_3 = 24.4H_2O + 10.5CO_2 + 4.3C_5H_7O_2N$

Sample Calculation for Theoretical Oxygen Demand:

1.52 g of POL (10) degraded in 25.5 hrs:

(1.52 ~~g~~/L) * (~~1 mol POL(10)~~/626 ~~g~~) * (21.5 ~~mol O2/1 mol POL (10)~~) * (32 ~~g~~/ ~~mole O2~~) * (1000 mg/g) * (1/25.5 ~~hr~~) * (1 ~~hr~~/60 min) = 1.09 mg/L-min

VITA

Keith La Torre was born in Huntington, NY. He graduated from Smoky Mountain High School (Sylva, NC) in 1991. Then, he graduated from the University of North Carolina at Chapel Hill in August, 1995, with a Bachelor of Science in Public Health (BSPH) in environmental science with a concentration in biology. He then interned with the Department of Energy in Oliver Springs, TN. There, he catalogued and researched the use of environmental remediation technologies. He then briefly researched plant molecular biology with Dr. Sabine Rundle at Western Carolina University (Cullowhee, NC). In August of 1996, Keith entered The University of Tennessee at Knoxville to work on his Master of Science degree in environmental engineering.