

AN ABSTRACT OF THE THESIS OF

David Bradley Roberts for the degree of Master of Science in Civil Engineering presented on October 10, 1997. Title: Down-Borehole Permeable Barrier Reactor: Verification of Complete Mineralization of Pentachlorophenol in a Sequential Anaerobic-Aerobic Process

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Laboratory scale batch bioassays were conducted to validate future results from a field scale demonstration of a down-borehole permeable barrier reactor. The batch bioassays used municipal anaerobic digester and aerobic activated sludge as the inoculum source in a sequential anaerobic - aerobic treatment process to provide evidence for complete mineralization of PCP in a contaminated aqueous environment.

Replicate bioassays were conducted in 250 mL amber serum bottles. During phase one, batch bioassays were maintained under anaerobic conditions and administered multiple additions of PCP, imitation vanilla flavoring, and nutrients to verify that bottles were behaving similarly and to determine a degradation pathway. The progress curves demonstrated similar PCP removal rates and showed transient production of 2,3,4,5-TeCP and accumulation of 3,4,5-TCP for all anaerobic bioassays. During phase two, the experimental set from phase one was divided and reactors spiked with non-labeled PCP were used to establish progress curves for duplicate reactors containing uniformly labeled pentachlorophenol (UL-¹⁴C-PCP). Reactors were maintained under anaerobic conditions until bioassays containing non-labeled PCP had completed reductive dechlorination of PCP to 3,4,5-TCP. After conversion of PCP to 3,4,5-TCP was observed, each bioassay was

supplied with excess oxygen, aerobic inoculum, imitation vanilla flavoring, nutrients, and deionized water.

Immediately following aerobic conversion, there was evidence for 3,4,5-TCP removal in reactors that initially contained non-labeled PCP. There was a simultaneous increase of activity in the headspace of reactors that initially contained UL-¹⁴C-PCP. The sequential anaerobic - aerobic process for the viable reactor sets demonstrated 58.6 percent conversion of UL-¹⁴C-PCP to ¹⁴CO₂ while the sterilized controls showed less than 0.2 percent mineralization. These results clearly demonstrate that a sequential anaerobic - aerobic process is an effective means of obtaining complete mineralization of PCP in a contaminated aqueous environment.

**Down-Borehole Permeable Barrier Reactor: Verification of Complete Mineralization of
Pentachlorophenol in a Sequential Anaerobic-Aerobic Process**

by

David Bradley Roberts

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David Bradley Roberts, Author

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PREFACE

This thesis is one in a series dedicated to the technological development of an *in situ* permeable barrier down-borehole reactor (See Table 5).

Chapter 1 includes a brief introduction to the down-borehole reactor concept and presents an overview of the physical characteristics of the demonstration site. Chapter 2 is the thesis written in manuscript format for submission to a technical journal for publication and chapter 3 provides suggestions for future work. The appendices list all pertinent experimental protocols, and embodies data in the form of graphs and tables.

Down-Borehole Permeable Barrier Reactor: Verification of Complete Mineralization of Pentachlorophenol in a Sequential Anaerobic-Aerobic Process.

1. INTRODUCTION

1.1. Research Objective:

Acknowledging the lack of published documentation surrounding sequential anaerobic - aerobic biological treatment processes and the need to validate an *in situ* field scale demonstration project, the work presented herein was undertaken to address one primary objective:

- To provide evidence for the complete mineralization of pentachlorophenol in an aqueous environment using a sequential anaerobic - aerobic biological treatment process

1.2. Down-Borehole Technology Concept:

The down-borehole concept was developed under the USEPA Western Region Hazardous Substance Research Center's technology transfer program. The intent of the transfer program is to expand laboratory-scale research technology into field-scale demonstrations and full-scale applications. The down-borehole concept applies laboratory studies with knowledge of physical site characteristics to provide relatively inexpensive and effective means of conducting an *in situ* bioremediation field-scale demonstration at a wood treating facility. The following discussion will furnish the reader with pertinent physical characteristics of the demonstration site and will present the down-borehole technology concept.

1.3. Demonstration-Site Physical Characteristics:

The following site characteristics were taken from the Remedial Investigation Report (Remediation Technologies Inc., 1994) for the L. D. McFarland Facility (Eugene, Oregon).

Geological stratigraphy: Soil at the L.D. McFarland Facility consists mainly of gravely fill underlain by native clay and silt layers. Below the clay and silt layers, a thin and fairly continuous layer of sand and silt is succeeded by a deeper and somewhat inconsistent layer of gravel and sand. The aquifer consists mainly of course grain soils that is bound by an upper layer of clay and silt ranging from 1.5 to 11 ft in thickness across the site (10 to 12 feet below ground surface). The lower portion of the aquifer seems to be contained by a sandy silt to dense silt layer with minor amounts of clay. This lower aquitard was pierced during 2 of the 8 initial deep borings (greater than 50 ft in depth) to reveal a clean deposit of sand and gravel.

Soil quality conditions: Contaminants found in the soils from boring samples consisted mainly of volatile organic carbons (VOCs), polynuclear aromatic hydrocarbons (PAHs), and pentachlorophenol (PCP). Two of the VOCs discovered on site, ethylbenzene and acetone, were detected at 0.3 mg/kg each in one of the 43 soil samples submitted for analysis. Xylenes, the only other VOCs detected on site were found at 0.8 mg/kg in two of the 43 samples submitted for analysis. PAHs and PCP were detected in about half of the soil samples submitted for analyses. Maximum PCP concentrations found were 47 mg/kg while maximum PAH concentrations were found to be as high as 6,500 mg/kg.

Site Hydrogeology: The hydrogeological investigation was limited to the northwest corner of the facility and off-site down-gradient regions. Regionally, the groundwater exists as an unconfined aquifer system. However, due to the upper clay and silt confining layer, the on-site aquifer is

confined during the winter months (high water) and unconfined during the summer months (low water). In general, regional groundwater flows northwest, but a shallow groundwater divide located in the northern portion of the plant produces flows to the north and south with some vertical gradients reported. An aquifer pumping test determined a hydraulic conductivity (K) ranging from 36.7 to 89.3 ft/day, storativity values (S) from 0.0017 to 0.0034, and a transmissivity (T) of 1830 to 4460 ft²/day (assuming an aquifer thickness of 50 ft) (Remediation Technologies, 1994).

Groundwater Quality: Groundwater contamination on site is primarily due to VOCs and semi-volatile organic carbon compounds (SVOCs). Light non-aqueous phase liquids (LNAPLs), observed over the northwest 1.5 acres of the site (beneath the wood treating area), were found in thicknesses ranging from inches to several feet. The principal components found in the LNAPL are xylenes, ethylbenzenes, toluene, PAHs, and PCP. PAHs have been observed to the northwest of the LNAPL occurrence and PCP has been observed to the north and to the west of the facility.

Although multiple groundwater contaminants are routinely monitored for, Remediation Technologies Inc. (1994) in concurrence with the USEPA determined that, "PCP was the primary indicator compound for the dissolved plume." In the interest of brevity, the following discussion will be limited to PCP.

No attempt was made to model the fate of dissolved PCP in the groundwater, however data acquired from October of 1990 through December of 1993 estimates that the plume extends 50 to 60 feet below the ground surface and travels in a generally circular shape having a diameter of approximately 4500 ft (outermost boundaries defined at a concentration of 1 µg/L for plume depth and breadth).

1.4. Introduction to the Down-Borehole Reactor:

The down-borehole reactor is a semi-passive, *in situ*, permeable barrier reactor technology. As a semi-passive technology, successful treatment relies on the natural groundwater gradient to pass contaminated water through the biological treatment zones within the reactor. However, the system is not completely passive since pumps are used for nutrient or substrate delivery and pressurized gas is used to maintain an appropriate treatment environment for the inoculum. The reactor is an *in situ* technology since all groundwater remediation occurs underground and is classified as a permeable barrier since the biological treatment zones are permeable to groundwater flow but act as a barrier for the organic contaminants. This down-borehole reactor was designed and fabricated as a means of bringing research knowledge to market in order to compete with other more costly and invasive remediation techniques.

1.5. Location of the Demonstration Site:

In early spring 1996, a cable tool rig was used to construct a 24 inch diameter well in the northwest corner of the L.D. McFarland Facility (down gradient of the retort chambers). The well was drilled 25 feet deep and was screened over a three foot interval to allow groundwater flow through the reactor treatment zones. Background concentrations of PCP in the groundwater at the demonstration site ranged from 0.5 mg/L to 1.5 mg/L between May, 1997 and September, 1997.

1.6. Rationale for the Down-Borehole Reactor:

Given the physical site characteristics, the conceptual design of the down-borehole reactor was dictated by one primary goal, to demonstrate *in situ* bioremediation of PCP (the primary indicator compound of the dissolved groundwater contaminant plume on site). In order to achieve this goal, multiple questions about biological treatment strategies were addressed under controlled research conditions. The following table represents a summary of research conducted to further develop the down-borehole technology.

Table 1: Research Conducted at OSU for the Development of the Permeable Barrier Down-Borehole Reactor

Research Subject	Author / Researcher	Year Completed
Pentachlorophenol (PCP) Biodegradation at Varying Temperatures and Low Substrate Concentrations	Tim Bricker	1993
Sorption of Chromium on Iron Coated Sand	Juei-Chu Tu	1993
PCP Reductive Dechlorination and the Significance of Temperature	Jason Cole	1994
Down - Borehole Permeable Barrier Reactor: Physical Development	James L. Brown	1995
Primary Substrate Selection for Aerobic Dichlorophenol Degradation	Peter Kaslik	1996
Bioremediation of PCP - Contaminated Well Cuttings in Laboratory and Full-Scale Processes	Mark Havighorst	Work in progress
Field Implementation of A Down - Borehole Permeable Barrier Reactor	Jason D. Cole	Work in progress
A Groundwater Aquifer Physical Model for the Study of Parameters that Influence PCP Degradation	Joseph B. Lotrario	Work in progress

1.7. Physical Configuration of the Down-Borehole Reactor:

The down-borehole reactor was designed and fabricated in four phases: the reactor body, the nutrient delivery system, the groundwater sampling system, and the reactor support and orientation structure. The first phase, design and fabrication of the reactor body, was completed during the Spring of 1995. The nutrient delivery system, groundwater sampling system, and

support structure were designed during the Fall of 1996 and fabricated during the winter and spring of 1997.

The reactor shell is a cylinder having a diameter of 23 inches and height of 42 inches. Stainless steel screen divides the interior of the reactor into three biological treatment zones, each of which is preceded by a mixing zone. The mixing zones contain the nutrient delivery system and a portion of the groundwater sampling ports.

The nutrient delivery system maintains anaerobic or aerobic conditions while ensuring complete mixing of the primary substrate, nutrients, and tracers (bromide) with the groundwater as it passes through the reactor. Aqueous solutions are delivered to the diffuser by above ground positive displacement piston pumps and are forced through the diffusers via compressed gas. The compressed gas is regulated by a solenoid valve attached to a timer (nitrogen for the anaerobic zones or oxygen for the aerobic zone). The mixing zones ensure that a uniform substrate and groundwater concentration enters the frontal plane of the biological treatment zone without disrupting natural groundwater flow.

The reactor employs anaerobic and aerobic mixed cultures in three biological treatment zones for the remediation of PCP-contaminated groundwater. The rationale for using a mixed culture and sequential treatment strategy is threefold. First, this treatment process ensures optimum degradation kinetics for highly chlorinated phenolic compounds. Anaerobic conditions provide the most rapid dechlorination rates for highly chlorinated phenols, but anaerobic degradation rates rapidly decrease with a decreasing degree of chlorination. Conversely, under aerobic conditions, degradation rates increase with a decrease in degree of chlorination. Second, PCP-degraders are ubiquitous so mixed cultures represent an easily accessible “off-the-shelf” source of inoculum.

The inoculum used is a mixture of municipal anaerobic digester sludge and municipal activated sludge. The inoculum is poured over borosilicate ceramic saddles (void space = 0.81) prior to commencing *in situ* studies to promote adequate attachment of the external cells. Third, due to symbiotic relationships exhibited by mixed cultures, they are much more likely to survive transfer into a foreign environment than cell masses which have been selected for on the basis of their remedial capabilities (i.e. pure cultures).

The sampling system uses pressurized gas plumbed to spring-loaded check valves to take spatially discrete ground water samples from the subsurface with time. Teflon lines (0.125 mm O.D.) connect check valves in the subsurface reactor to above ground ball valves and pressurized gas cylinders. When the lines are pressurized, the check valves remain closed. As the line is "bled" to atmospheric pressure and then quickly repressurized, the subsurface valve opens, allowing a small sample of water (less than 1 mL) into the line, and then recloses as pressure builds. The in line pressure then forces the groundwater sample to the surface where it is collected and stored in a cooler for analysis.

The support structure is a mobile system that permits field workers to raise, lower, and modify the orientation of the reactor in the well as needed. After the reactor is lowered into the desired location and rotated for proper orientation, the reactor is suspended in place using a load bearing plate with a free moving bushing to distribute the load over the well casing.

1.8. Discussion:

The driving force behind this study was to verify that a field-scale application that uses a sequential anaerobic - aerobic treatment process can result in complete mineralization of pentachlorophenol. The following is a list of physical parameters that vary significantly between

the laboratory-scale research and the future field-scale demonstration project. These parameters were not ignored during experimentation, but simplified methods were dictated due to the nature of restrictions placed upon radiochemical work.

- Groundwater temperature: The ground water temperature at the site is $15 \pm 5^{\circ}\text{C}$ while temperatures in the laboratory scale demonstration were $21 \pm 4^{\circ}\text{C}$.
- General water chemistry found on site (Appendix H): Multiple inorganic compounds are found in the ground water. Some of these (such as sulfate and nitrate) can serve as electron donors under anaerobic conditions, affecting PCP degradation rates. Sulfate and nitrate were not added to laboratory experiments reported herein.
- Other PAHs, dioxins, furans, and semivolatile organic compounds are present in the groundwater and may present toxicity problems during technology demonstration.
- The down-borehole reactor will be a continuous flow system while laboratory research was conducted using batch reactors.

2. EXPERIMENTAL FINDINGS AND RELATED INFORMATION

2.1. Introduction:

2.1.1. Chemical Properties of Pentachlorophenol:

Pentachlorophenol (PCP) is a recalcitrant chlorinated aromatic compound. Its anti-fungal and anti-biological properties combined with chemical stability have made it the long-standing wood preserving agent of choice in the lumber industry. From 1987 to 1993, PCP released to the environment totaled nearly 100,000 pounds making it present on at least 260 of 1416 National Priorities List Sites (EPA Toxic Chemical Release Inventory, 1995). PCP is a class B2 carcinogen and exposure has been linked to membrane irritation, dysfunction of the liver, kidneys, blood, lungs, nervous system, immune system, and gastrointestinal tract (The Agency for Toxic Substances and Disease Registry, 1994). Consequently, the EPA has established a Maximum Concentration Level (MCL) of 0.001 mg/L and a Maximum Concentration Level Goal (MCLG) of zero mg/L for drinking water standards.

Due to a growing number of PCP-contaminated sites and the prohibitive expense encountered when employing full-scale clean up efforts, biological processes have become an attractive alternative for the remediation of PCP-contaminated soil and water systems. Studies that examine the reductive dechlorination of PCP under anaerobic conditions or the oxidation of PCP under aerobic conditions are well documented in the literature, but only a modest portion of these reports provide evidence for metabolic production of carbon dioxide or methane from the degradation of PCP (e.g. mineralization). All documents referenced herein, that provide evidence for mineralization, employ radiotracer analysis to verify that PCP had been fully reduced or oxidized under anaerobic or aerobic conditions.

2.1.2. Pentachlorophenol Mineralization:

To date, the majority of the PCP mineralization work presented in the literature has been conducted under aerobic conditions using a pure culture as a source of inoculum. These laboratory-scale exercises have been quite successful, and in fact mineralization rates as high as 83 percent within just 115 hours of incubation have been reported using Strains of *Flavobacterium* (Saber and Crawford, 1985). Subsequent pure culture work was conducted employing immobilized *Rhodococci* strains to achieve 40 percent mineralization in two weeks (Valo et al., 1989) and free or encapsulated *Pseudomonas* sp. for up to 65 percent mineralization in as little as one day (Cassidy et al., 1997).

Undisputedly, pure culture work in the laboratory is the most logical method for obtaining repeatable scientific results, but the ultimate goal for conducting environmental bioremediation research should be field implementation. Almost without exception, successful biological field applications employ complex consortia or indigenous microbial populations, not pure cultures. Yet there is little published evidence for mineralization of PCP using a mixed culture. Tranvik et al. (1990) evaluated the ability of indigenous lake microbes to degrade xenobiotic compounds (PCP, 2,4,5-TCP, and 3,4-DCP) and as might be expected, presented a mineralization rate considerably slower than pure culture work. Only 1-6 percent of total chlorophenols were mineralized within 54 hours of *in situ* incubation.

There are also few references to PCP mineralization under anaerobic conditions. Examples of successful studies which have demonstrated complete anaerobic PCP mineralization were first reported by Mikesell and Boyd (1986) and later by Kennes et al. (1995). In contrast to most aerobic work reported, neither groups used pure cultures to demonstrate anaerobic mineralization of PCP. Instead, Mikesell and Boyd (1986) employed a monochlorophenol-acclimated anaerobic

digester sludge and Kennes et al. (1995) used methanogenic PCP-degrading granular sludge. Mikesell and Boyd (1986) demonstrated up to 66 percent PCP mineralization (including a carbonate recovery factor and calculated stoichiometric amount of $^{14}\text{CH}_4$) within two months of incubation while Kennes et al. (1995) were less concerned with demonstrating mineralization than with investigating the role that sulfate-reducing microorganisms play in anaerobic reductive dechlorination and mineralization of PCP. Kennes et al. (1995) presented no overall percentage of mineralization, but developed a statistically significant correlation for chloride ion release with $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$ production under conditions that select for sulfate reducing microorganisms.

The work presented here was conducted using a municipal wastewater consortium in a sequential anaerobic-aerobic process to provide evidence for complete mineralization of PCP in an aqueous environment. The motivation for using this type of treatment methodology is two fold. First, degradation kinetics are optimized in a sequential system (highly chlorinated phenols are most readily dechlorinated under anaerobic conditions while lower chlorinated metabolites are quickly hydroxylated and subsequently dechlorinated under aerobic conditions). Second, since PCP-degrading microorganisms are ubiquitous, it is our contention that sequential systems that employ complex consortia offer the greatest likelihood for success in full-scale field applications.

Although sequential anaerobic - aerobic systems offer exceptional potential for the remediation of chlorinated phenols, almost no applications of sequential treatment strategies have been documented. A review yielded three publications which have employed sequential biological processes for the treatment of chlorinated phenols (Armenante et al., 1992; Fahmy et al., 1994; and Bhattacharya et al., 1995), however none provided evidence for PCP mineralization.

2.2. Methods and Materials:

The mineralization of PCP in a sequential anaerobic-aerobic process was evaluated in a two-phase experiment. During the non-labeled phase, six batch replicate bioassays were conducted in serum bottles consisting of four active bottles and two controls. Each replicate was given multiple additions of reagent grade pentachlorophenol (PCP) to establish biotransformation pathways and to verify that bioassays behaved similarly. During the second phase, mineralization was verified. The bottle set from the non-labeled phase was divided and uniformly labeled pentachlorophenol (UL-¹⁴C-PCP, SIGMA Chemical Company, St. Louis, MO) was added to half the bottles. The remaining bottles in the set received an equivalent amount of non-labeled PCP. The chlorophenol additions resulted in a final concentration of 1.9 μM (0.5 mg/L) PCP or 1.9 μM (0.5 mg/L) UL-¹⁴C-PCP and an activity of 1.95 μCi. After complete transformation of PCP to 3,4,5-TCP was observed in the non-labeled active replicates, the reactors were converted to aerobic conditions and progress curves were further developed using gas chromatography and liquid scintillation analysis as applicable. The control reactors received one addition of PCP at the beginning of each phase. The first addition, for the non-labeled phase resulted in a concentration of 1.9 μM (0.5 mg/L) PCP. For the mineralization phase, the initial concentration was increased from 1.9 μM (0.5 mg/L) to 2.6 μM (0.7 mg/L) by adding PCP or UL-¹⁴C-PCP (0.60 μCi).

2.2.1. Inoculum:

Inoculum was obtained from the Corvallis Municipal Wastewater Treatment Facility on October 3, 1995. Return activated sludge and anaerobic digester sludge were mixed in equal amounts expressed as volatile suspended solids (VSS) and stored at room temperature. Subsequently, the mixture of cells was divided. Half of the cells were held under anaerobic conditions and

acclimated to PCP by repeated additions resulting in 0.75 μM (0.2 mg/L) PCP. The other half of the cells was administered a continuous supply of compressed air and was initially acclimated to 3,4-DCP and 3,5-DCP at 3.1 μM (0.5 mg/L) each and later acclimated to 3,4,5-TCP at 2.6 μM (0.5 mg/L). Both carboys received monthly additions of imitation vanilla flavoring at less than 100 mg COD/L and nutrients as recommended by Owen et al. (1979). All values for Total Solids and Total Volatile Solids reported were determined according to methods 2540B and 2540E, Standard Methods for the Examination of Water and Wastewater, 18th Edition (American Public Health Association, 1992).

2.2.2. Experimental Design:

Anaerobic Reactor Design: Anaerobic reactors consisted of six 250 mL amber Boston round septa bottles (Fisher Scientific, Santa Clara, CA) containing: 3.2 g/L Total Volatile Solids (TVS) anaerobic inocula, 1.9 μM (0.5 mg/L) PCP or 1.9 μM (0.5 mg/L) UL- ^{14}C -PCP, 250 mg COD/L imitation vanilla flavoring, nutrients as suggested by Owen et al. (1979), and sufficient deoxygenated deionized water (D.I.) to result in a final volume of 100 mL. All transfers were conducted under purified nitrogen gas. The controls were set up identically to the active replicates with the substitution of 3.2 g/L sterilized autoclaved cells in place of viable cells. Bioassays were maintained at $21 \pm 4^\circ\text{C}$. The experiment was later repeated using a higher substrate concentration of 400 mg COD/L imitation vanilla.

Aerobic Reactor Design: Upon conversion of PCP to dechlorinated metabolites, the pH was adjusted to 6.3 and the following components were added to each anaerobic reactor to yield the following concentrations: 200 mg COD/L imitation vanilla, 1.3 g/L TVS aerobic inocula, nutrients in excess (Owen et al. 1979), and required volume of D.I. water to result in a total

addition of 50 mL. The reactors were sealed with new septa and purged with 99.99 percent pure oxygen for 15 headspace volumes. A chlorophenol assay was conducted for non-labeled bottles prior to and after aerobic conversion to account for chlorophenol reduction due to dilution, volatilization, and sorption. Likewise, liquid scintillation analyses were performed before and after conversion for the ^{14}C -labeled replicates.

2.2.3. Analytical Procedures:

2.2.3.1. *Gas Chromatography Analysis:*

Reactors were monitored using a miniaturized hexane extraction method developed by Voss et al. (1981), and modified by Perkins (1992) and Smith and Woods (1993). The method calls for mixing 100 μL aqueous samples with 1 mL of a reagent containing 30.4 g/L K_2CO_3 , 500 mg/L 2,4,6-tribromophenol (an internal standard), and 100 μL of acetic anhydride. The 100 μL samples and reagents are added to a borosilicate glass test tube which is sealed with an air-tight Teflon[®]-lined cap and placed on a wrist shaker for 20 minutes. Following mixing, 1 mL of HPLC grade hexane is added to each test tube which is resealed and returned to the wrist shaker for an additional twenty minutes. After shaking is complete, the hexane fraction is removed and placed into vials capped with Teflon[®]-faced silicon septa for gas chromatography analysis.

A Hewlett-Packard-6890 series II gas chromatograph equipped with a ^{63}Ni electron capture detector and a J&W Scientific DB-5MS 30m column (J&W Scientific, Folsom, CA) was used for chlorophenol quantification. Hewlett-Packard Chemstation software: Rev.A.05.01 [273][®] was responsible for system control and was programmed as follows: An initial oven temperature of 40°C was held for 1 minute then increased at a rate of 25°C/min to achieve a temperature of 140°C. The oven temperature was further increased from 140°C to 250°C at a rate of 10°C/min

and held for 5 minutes while injector and detector temperatures were kept at a constant 250°C and 350°C respectively. Helium served as the carrier gas with an initial flow of 2.00 mL/min for 14.00 min. The flow was then increased at 4.00 mL/min for a final flowrate of 4.00 mL/min for 7 min. A 95:5 mix of Ar:CH₄ was used for detector auxiliary gas at a flow rate of 60 mL/min. Custom chlorophenol standards (Ultra Scientific, North Kingstown, RI) were used to create standard curves for instrument calibration.

2.2.3.2. *Liquid Scintillation Analysis:*

Two liquid scintillation assays were developed for monitoring reactors spiked with radiochemicals. The first method was used to account for activity in the reactor liquid while the second method was developed to determine activity in the headspace.

Aqueous sample preparation: To ensure uniform samples, each reactor was shaken for 10 seconds prior to sample extraction. Aqueous samples(10-20 µL) were drawn from each reactor (volume was dependent on desired statistical accuracy). The sample was then injected below the surface of 10 mL of ScintiSafe Econo 2 scintillation cocktail contained in 20 mL borosilicate scintillation vials with polyethylene caps. Cocktail and vials were acquired from Fisher Scientific (Santa Clara, CA).

Headspace sample preparation: The headspace of each labeled reactor was replaced with 15 headspace volumes of nitrogen gas during the anaerobic phase or an equivalent quantity of oxygen gas during the aerobic phase. A headspace trapping mechanism was designed and built to pass effluent gasses directly through six 20 mL scintillation vials plumbed in series with 3.75 mm O.D. TFE tubing (Appendix I). Each vial contained 5 mL of Carbo-Sorb[®]E radioactive carbon dioxide

absorber which received 10 mL of Permafluor[®] E⁺ scintillation cocktail prior to counting (Packard Instrument Co., Meriden, CT).

Aqueous and headspace samples were quantified using a Packard Tri-Carb Model 2500 TR/AB liquid scintillation analyzer. Samples were counted for 60 minutes or until sufficient counts were acquired to achieve a number of decay events ± 2 percent with a 95.5 percent confidence level.

Counting efficiency was monitored using Packard's transformed external standard spectrum (TSIE) technique. Self-Normalization and instrument Calibration (SNC) was performed every 23 hours using an unquenched ¹⁴C standard with Packard's Instrument Performance Assessment (IPA) protocol. Method blanks were prepared for each sample period and served as background counts.

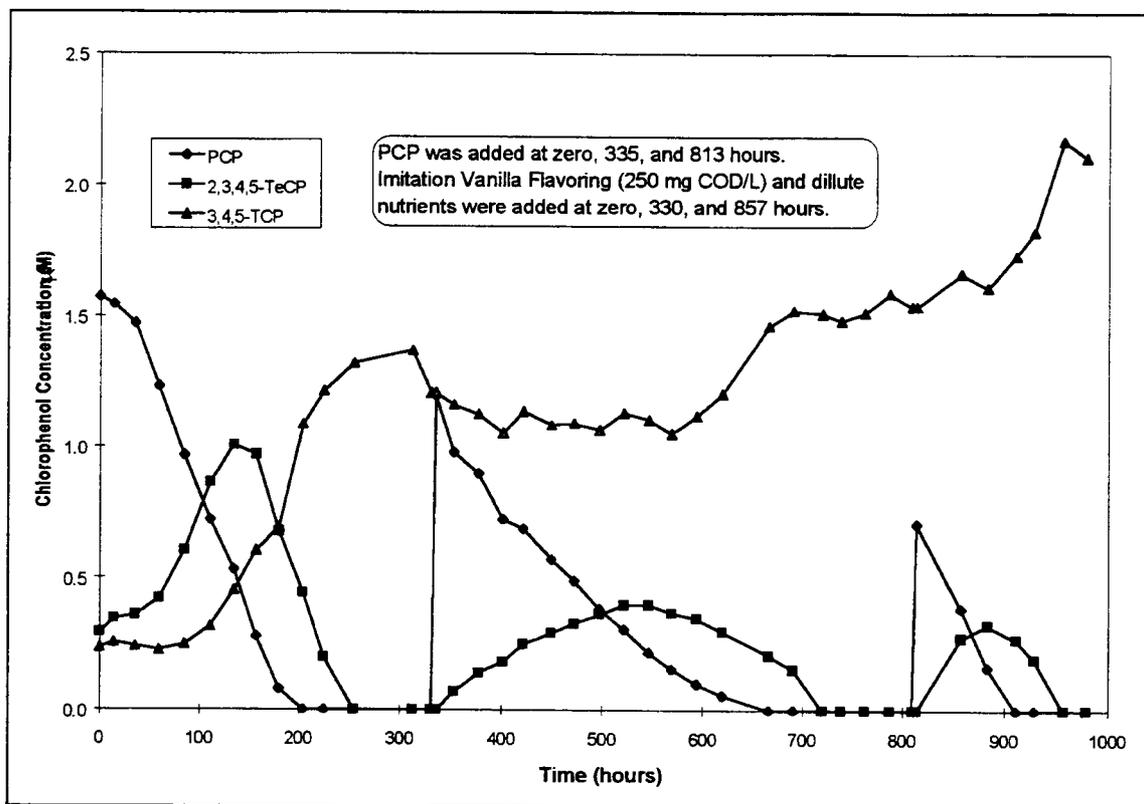
2.3. Results and Discussion:

The focus of our work was to demonstrate mineralization of PCP in an aqueous environment using a sequential anaerobic-aerobic process. Preliminary studies were conducted using non-labeled PCP and gas chromatographic analyses to characterize removal under anaerobic and aerobic conditions. Mineralization experiments required the use of UL-¹⁴C-PCP, which was monitored using liquid scintillation analysis.

2.3.1. Anaerobic Progress Curves:

Progress curves were developed for anaerobic batch bioassays in which imitation vanilla flavoring served as the electron donor (Figure 2.1). PCP was reductively dechlorinated at the ortho positions, producing 2,3,4,5-TeCP transiently and accumulating 3,4,5-TCP. This pathway was observed for each of three PCP additions (0, 335, and 813 hours). At each addition of imitation vanilla flavoring, a step increase in the concentration of 3,4,5-TCP was observed (data not shown). In all cases, the 3,4,5-TCP concentrations returned to previous levels by the next sampling period. These transient 3,4,5-TCP concentrations may have been due to the surfactant-like characteristics of the aromatic components of imitation vanilla flavoring (Khodadoust et al., 1994). The presence of 2,3,4,5-TeCP (0.31 μM) and 3,4,5-TCP (0.24 μM) at time zero was due to residual concentrations in the inoculum.

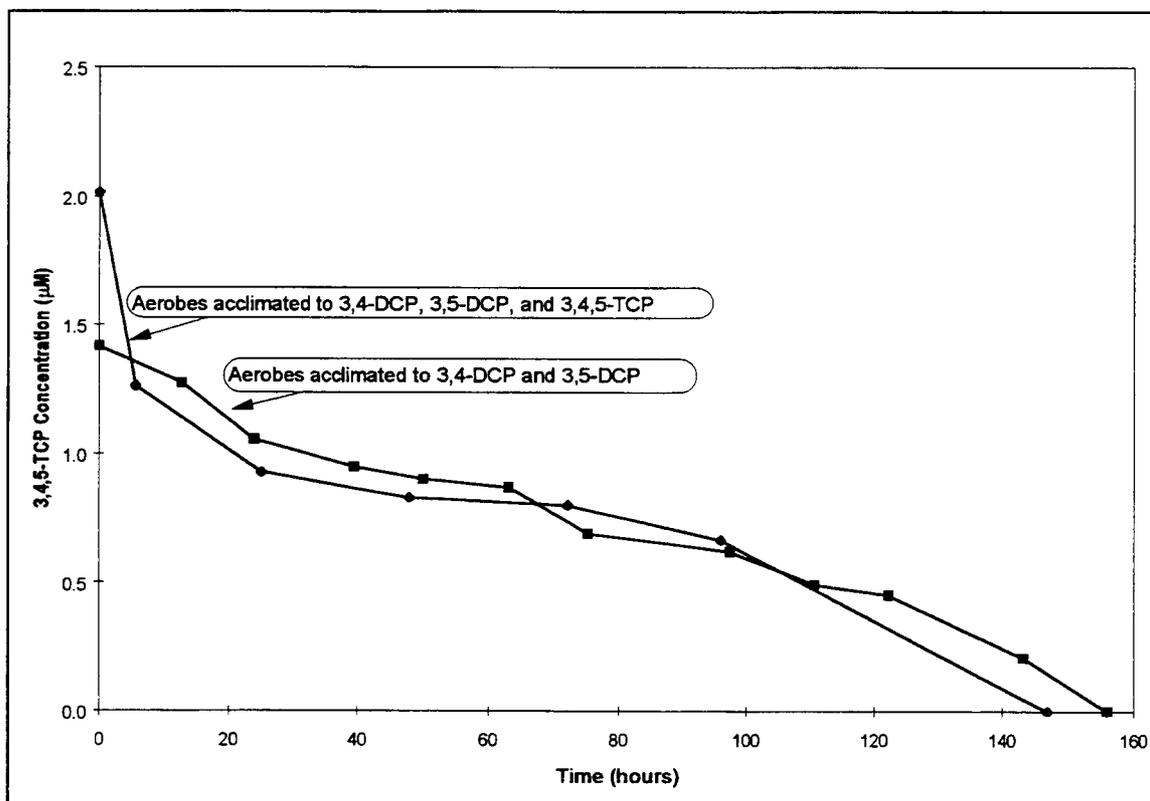
Figure 2.1 Anaerobic Transformation of PCP in Batch Bioassays



2.3.2. Aerobic Progress Curves:

Because 3,4,5-TCP accumulated under anaerobic conditions, its removal was evaluated in aerobic batch bioassays. Imitation vanilla flavoring at 250 mg COD/L served as the primary substrate in the presence of excess oxygen and nutrients (Figure 2.2). The first bioassay employing inocula acclimated to 3,4-DCP and 3,5-DCP (3.1 µM each) demonstrated the ability to remove 3,4,5-TCP at 1.41 µM (0.28 mg/L) within 156 hours. Studies were repeated using inocula acclimated to 3,4-DCP(3.1 µM), 3,5-DCP(3.1 µM), and 3,4,5-TCP(2.6 µM), resulting in the removal of 2.0 µM 3,4,5-TCP in 143 hours. These similar 3,4,5-TCP removal rates suggested that acclimation of inoculum to 3,4,5-TCP does not significantly affect 3,4,5-TCP degradation kinetics by these mixed consortia under aerobic conditions.

Figure 2.2 Aerobic Removal of 3,4,5-TCP in Batch Bioassays

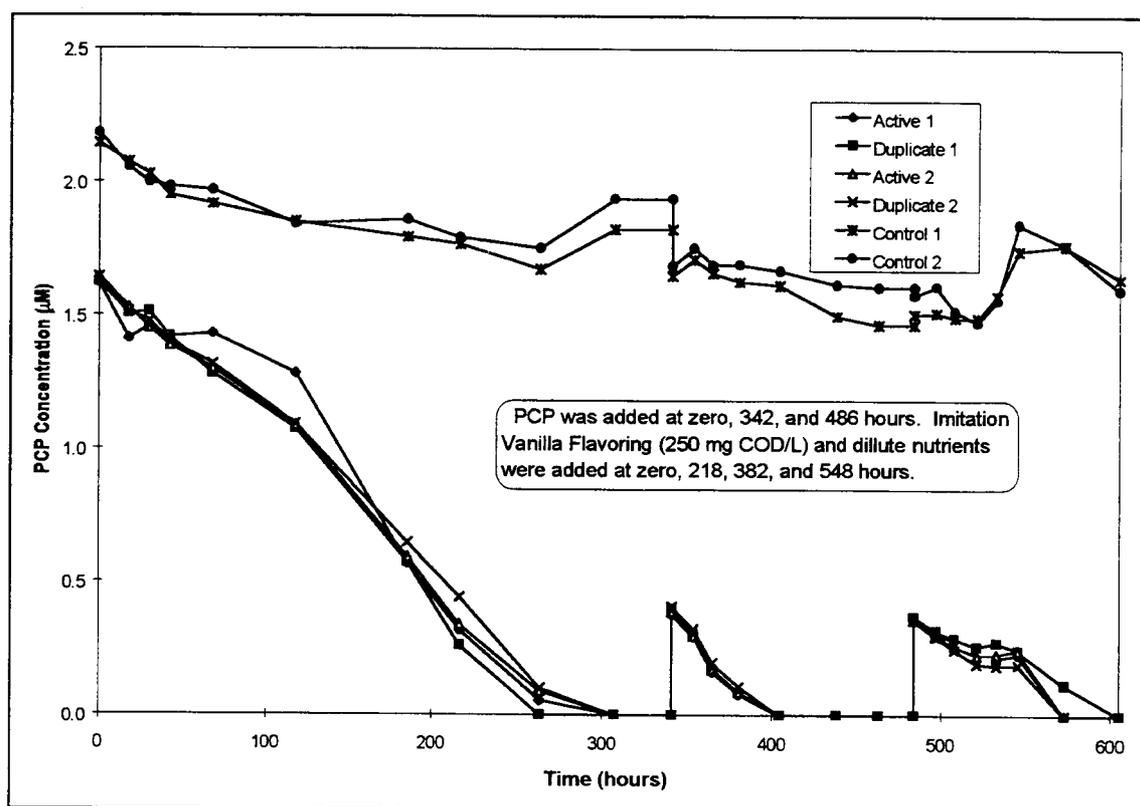


After completing preliminary anaerobic and aerobic studies, a system was implemented to provide evidence for mineralization under sequential anaerobic - aerobic conditions. These experiments required use of ^{14}C -labeled PCP (UL- ^{14}C -PCP) limiting analyses to liquid scintillation. Since liquid scintillation was ineffective at providing information regarding anaerobic transformation activity, duplicate reactors were created with reagent grade non-labeled PCP. These duplicate reactors made it possible to observe reductive dechlorination products and to determine the appropriate time frame for conversion of the reactors to aerobic conditions.

2.3.3. PCP Removal Rates Among Duplicate Reactors:

Anaerobic reactors and their duplicates were given three injections of PCP to demonstrate that the reactors were behaving similarly (Figure 2.3). These additions showed that some variability of the PCP removal rate was likely to occur from one PCP addition to the next, but that all bioassays responded to changes of environmental conditions (i.e. temperature, substrate concentration, nutrient levels, etc.) almost identically (Figure 2.3). This uniform response made it reasonable to predict processes in reactors spiked with UL-¹⁴C-PCP using progress curves developed from duplicates spiked with non-labeled PCP.

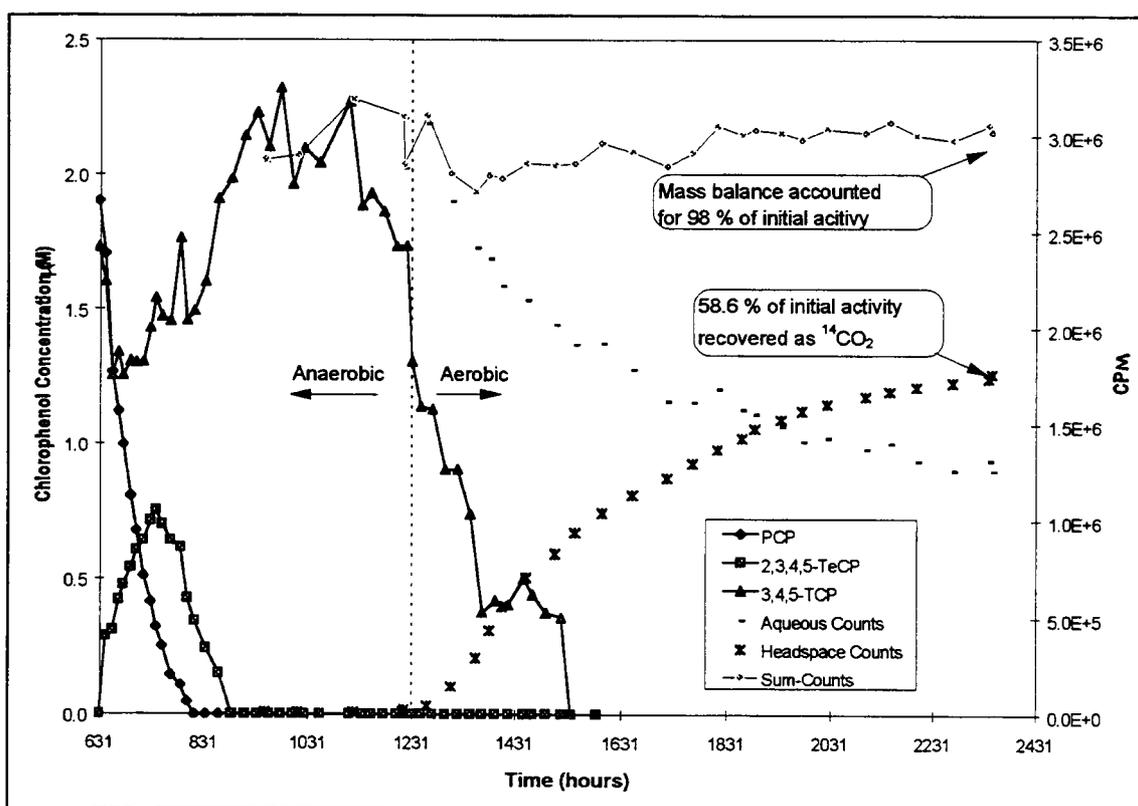
Figure 2.3 Similar PCP Removal Rates Demonstrated in Anaerobic Batch Bioassays



The fourth PCP addition at 631 hours marked the beginning of the mineralization phase (Figures 2.4 and 2.5). The experimental set was divided into two parts. Half the viable reactors were

given $1.85 \mu\text{M}$ PCP (0.50 mg/L) while the remaining viable reactors were given an equivalent dose of $\text{UL-}^{14}\text{C-PCP}$ ($1.95 \mu\text{Ci/bottle}$). Complete PCP removal was observed for the non-labeled reactors within 304 hours. All reactors were kept under anaerobic conditions for 336 hours following complete conversion of PCP to 3,4,5-TCP to ensure anaerobic processes were complete in the labeled reactors (Figure 2.4).

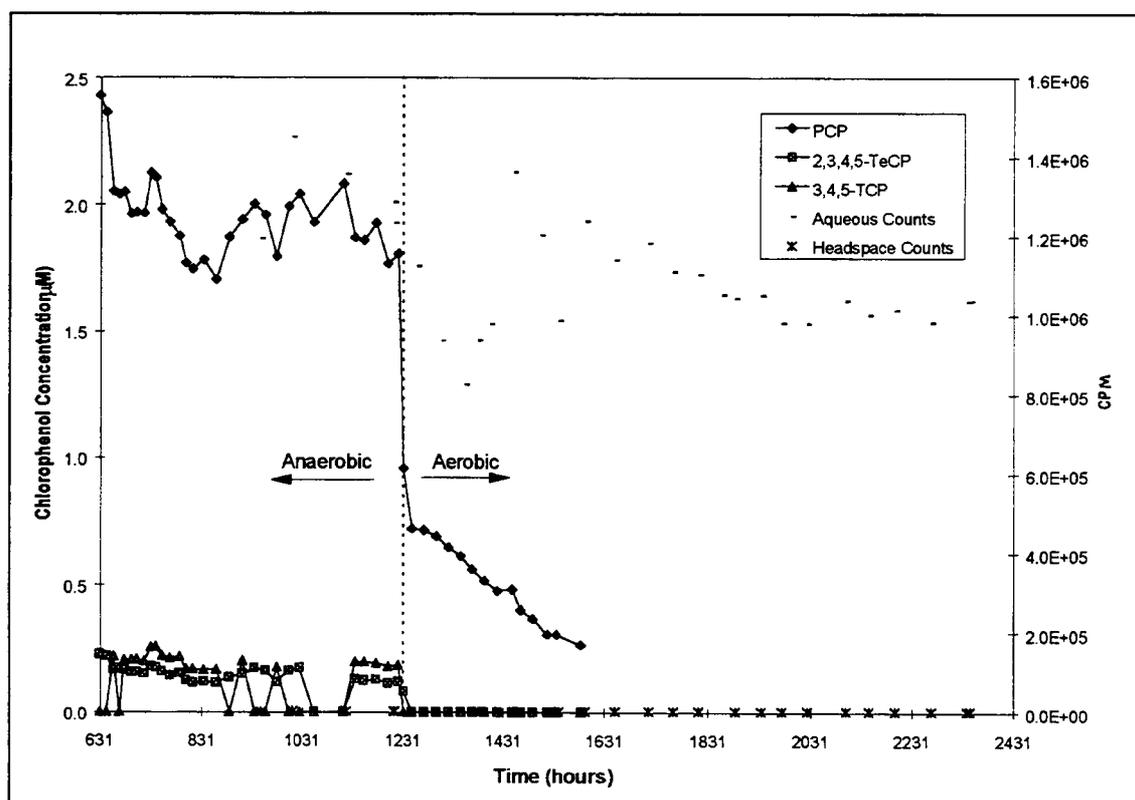
Figure 2.4 Evidence for Mineralization in Labeled and Non-labeled Batch Bioassays



Sterilized controls were dosed with PCP at time zero and again at 631 hours (Figures 2.3 and 2.5). The first dose resulted in a concentration of $2.16 \mu\text{M}$ PCP (0.65 mg/L) which fell to $1.62 \mu\text{M}$ PCP (0.43 mg/L) within 607 hours. This apparent reduction of PCP concentration in the controls was not accompanied by production of dechlorinated metabolites or removal of residual 2,3,4,5-TeCP or 3,4,5-TCP. At 631 hours, the PCP concentration in one control was brought to

2.43 μM PCP (0.65 mg/L) while the other control was given an equal addition of $\text{UL-}^{14}\text{C-PCP}$ resulting in 0.60 μCi (Figure 2.5). Once again, the PCP concentration fell during the anaerobic period (631 to 1231 hours). PCP removal was not accompanied by a decrease in the concentration of residual 2,3,4,5-TeCP or 3,4,5-TCP or a production of dechlorinated metabolites, indicating that biodegradation did not occur in the sterilized controls under anaerobic conditions.

Figure 2.5 Evidence for Non-mineralization in Labeled and Non-labeled Control Batch Bioassays



2.3.4. Evidence for Mineralization:

At 1230 hours, the sterilized controls and active assays containing either labeled or non-labeled PCP were converted to aerobic conditions (Figures 2.4 and 2.5). An aerobic inoculum acclimated to 3,4-DCP and 3,5-DCP was added, increasing the volume in the bottles from 100 mL to 150 mL. Imitation vanilla flavoring at 200 mg COD/L and dilute nutrients were added prior to exchanging the headspace with 15 volumes of pure oxygen.

2.3.5. No Evidence for Mineralization in Controls:

Immediately upon conversion to aerobic conditions, there was evidence for removal of 3,4,5-TCP within the non-labeled active bottles. Within the bottles containing UL-¹⁴C-PCP, a reduction of the activity in the liquid phase was accompanied by a simultaneous increase of activity in the gas phase (Figure 2.4). This general trend was observed at varying degrees for replicate reactors containing UL-¹⁴C-PCP (data not shown). Conversely, the sterilized controls that were established and maintained under identical conditions did not demonstrate production of 3,4,5-TCP from PCP, nor was transformation of activity from the aqueous phase to the headspace observed upon aerobic conversion (Figure 2.5). Headspace activity for all sample periods demonstrating significant counts was verified to be ¹⁴CO₂ using BaCl₂ precipitation (Mikesell and Boyd, 1992) (Appendix F).

2.4. Summary and Conclusions:

Removal of 3,4,5-TCP under aerobic conditions for bottles containing non-labeled PCP coupled with simultaneous ¹⁴CO₂ production in the labeled bottles verified that complete mineralization had occurred in the sequential anaerobic-aerobic batch bioassays (Figure 2.4). Counts appeared in the reactor headspaces directly following aerobic conversion and proportionately increased with 3,4,5-TCP removal reaching a maximum mineralization rate of 4.4 percent/day. Reactors

containing UL-¹⁴C-PCP were acidified at 2,346 hours to release H₂¹⁴CO₃ from solution.

Acidification resulted in an increase of 0.7 percent of initial activity. This indicates conversion of 58.6 percent of the UL-¹⁴C-PCP to ¹⁴CO₂.

Directly following aerobic conversion during the mineralization phase, PCP removal was observed in the non-labeled sterilized control (Figure 2.5). Several explanations exist for the decline in PCP concentration under aerobic conditions. Possibilities range from insufficient sterilization of the inocula to polymerization of imitation vanilla flavoring with PCP. Although PCP removal in the killed control was observed upon aerobic conversion, no significant activity was detected in the headspace of the duplicate control containing UL-¹⁴C-PCP for the duration of the experiment (0.17 % of initial counts over 2,346 hours). No activity in the control headspace confirmed that mineralization of PCP had not occurred in the sterilized control reactors.

For each active labeled batch bioassay presented in Table 2, a non-labeled duplicate demonstrated complete removal of PCP in the sequential anaerobic-aerobic treatment process. The non-labeled duplicates for both controls demonstrated no anaerobic transformation of PCP, but did demonstrate PCP removal under aerobic conditions. The aerobic PCP removal in the non-labeled controls was not accompanied by evidence for mineralization in their labeled duplicates.

Table 2: Mineralization of PCP in Sequential Anaerobic-Aerobic Reactors

	SEQUENTIAL LABELED REACTORS				
	Active 1	Active 2	Active 3	Control 1	Control 2
% mineralization	58.6	9.7	3.8	0.2	0.1
% activity recovered	98	98	93	85	100

2.5. Engineering Significance:

The purpose for undertaking this work was to verify that an *in situ* down-borehole permeable barrier reactor employing a sequential anaerobic - aerobic process can result in the complete mineralization of PCP. As with all experiments conducted in the laboratory, significant physical differences exist between the mineralization study and the field demonstration project (Table 3).

Table 3: Physical Parameters of the Mineralization Study and Field Application

PARAMETER	MINERALIZATION STUDY	DOWN-BOREHOLE REACTOR
Temperature (°C)	21 ± 4	15 ± 5
Reactor Configuration	Batch	Continuous flow
Aqueous pentachlorophenol concentration (µM)	1.9 (peak), 5.6 (total) (reagent grade)	1.9-5.6* (industrial grade)
Substrate concentration (mg COD/L)	200-250	50-100
Cell Mass concentration (total solids / volatile solids: mg/L)	420-630 / 110-240	Not available, but estimated to be higher than concentrations used in the mineralization study
Source of inoculum	PCP-acclimated mix of anaerobic and aerobic Corvallis municipal wastewater treatment sludge.	Unacclimated mix of anaerobic and aerobic Corvallis municipal wastewater treatment sludge.
Degradation period	71.4 days	Approximately 2 days

* Dioxins, Furans, and VOCs also found in the site groundwater samples

Several of the parameters listed above may have a significant effect on the outcome of the field demonstration of the down-borehole permeable barrier reactor. Lower groundwater temperatures and shorter reaction times for the site study may be problematic, however Cole et al. (1996) demonstrated PCP transformation in a packed column with water temperatures as low as 10 °C. The down-borehole reactor is subjected to a groundwater gradient representing a continuous flow configuration. Continuous flow reactors select for microbes that best adapt to the surrounding environmental conditions (i.e. lower temperatures, shorter reaction periods, and additional

groundwater contaminants). In addition, the anticipated cell mass concentrations in the bore-hole reactor will be significantly higher than concentrations in the mineralization study. Higher cell mass concentrations will require the same acclimation period but should demonstrate faster PCP removal rates.

Although this study can not predict the likelihood for success of the down-borehole reactor, it does verify that observed chlorophenol removal in the aerobic treatment zone can be interpreted as complete mineralization.

2.6. Acknowledgment:

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3. SUGGESTIONS FOR FUTURE RESEARCH

This study was completed as part of the technology development for an *in situ* permeable barrier down-borehole reactor. To successfully implement the down-borehole technology, the following questions should be answered.

1. Is it possible to employ a sequential anaerobic - aerobic process at one pH? If not, what is the best means of controlling the pH *in situ*.
2. What effect will the other contaminants found in the site groundwater have on the degradation process (inorganics, nutrients, dioxins, furans, ect.)? Another batch bioassay should be conducted that investigates a sequential anaerobic - aerobic process using groundwater from the site.
3. Since PCP-degraders are ubiquitous, would another source of inoculum be more likely to adapt to site conditions? Microbes found in the soils of surface water ponds at the site might be a good alternative.
4. What is the toxicity of the components of imitation vanilla flavoring (guaiacol, ethyl vanillin, propylene glycol, benzoate)? Would microorganisms utilize imitation vanilla flavoring more effectively without one of the components?
5. Another batch bioassay should be conducted to verify that sequential anaerobic - aerobic treatment achieves the greatest PCP removal rates (in comparison to strictly anaerobic or

strictly aerobic treatment processes). The down-borehole reactor can be readily modified to operate under strictly aerobic or strictly anaerobic conditions.

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APPENDICES

Appendix A

Experimental Protocol for Sequential Batch Reactors

Objective:

To verify complete mineralization of uniformly labeled pentachlorophenol (UL-¹⁴C-PCP) in a sequential anaerobic-aerobic batch reactor system.

Analyses:

Chlorophenol Assay

Permanent Gas Assay

TS/TVS Assay

Liquid Scintillation Assay

Materials:

Magnetic stir plate

2 x magnetic stir bar

12 x 250 mL amber glass serum bottles with Teflon[®]-coated silicon septa and screw caps

Aluminum foil

Compressed nitrogen and hydrogen cylinders with regulators and purification furnace

Compressed oxygen cylinder with regulator

1 x 25 mL x 1/10 glass pipette with bulb

1 x 1 mL glass ground syringe with 22 gage needle

1 x 10 mL glass ground syringe with 22 gage needle

1 x 500 mL wash bottle with D.I. water

2 x 500 mL Erlenmeyer flask with sponge stopper or aluminum foil

1 x 50 mL graduated cylinder

2 x 500 mL beaker

1 x 500 mL volumetric flask with glass ground stopper

1 x 200 mL volumetric flask with glass ground stopper

1 x 250 mL squeeze bottle

Nitrile gloves

Parafilm® laboratory film

Fisher brand recording thermometer

Chemicals:

Imitation vanilla flavoring

Media (Owen et al., 1979): S3, S4, S7

Deionized water (D.I.)

pentachlorophenol (PCP) stock solution at 20 mg/L

100 μ Ci of uniformly labeled pentachlorophenol (UL-¹⁴C-PCP) stock solution at 12.8 mg/L

Procedure:

- I. Stock Solutions: prepare according to the following directions and concentrations.
 - A. Stock PCP solution: Add 10 mg of PCP to 500 mL D.I. water in a 500 mL volumetric flask to prepare a stock solution of PCP at 20 mg/L. This concentration exceeds the saturation limit and may require an increase in pH and some mixing to drive the PCP into solution. Place a small, clean magnetic stir bar into solution, cap with a glass ground stopper, and wrap the top of the flask using Parafilm® Laboratory film. Place the solution on a magnetic stir plate,

cover and set speed at 8 to mix overnight. After mixing is complete, remove the magnetic stir bar, recap and wrap the flask using Parafilm[®] Laboratory film.

Store the stock solution away from UV light source to increase shelf life.

- B. Stock UL-¹⁴C-PCP solution: Complete all transfers in the Environmental Health and Safety approved fume hood located in Merryfield 103. Stock dilution carried out using a 200 mL volumetric flask. Acquire 100 μ Ci of dry uniformly labeled ¹⁴C-PCP with a specific activity of 10.4 mCi/mmol (supplied by SIGMA Chemical Company, Lot Number 037H9214). The sample is packed for shipping in a 2 mL conical vial with a Teflon[®]-coated butyl rubber septa. Use the 1 mL Luer Lock glass ground syringe to perform numerous 1 mL extractions of D.I. water from the sealed conical vial containing the UL-¹⁴C-PCP dry sample. Once there is no visible chemical remaining in the vial, uncap and rinse septa and vial thoroughly with D.I. water. Finally, fill the volumetric flask to 200 mL, cap, and wrap with Parafilm[®] laboratory film. Invert multiple times and store overnight to ensure complete mixing. Transfer stock solution to an amber 250 mL vial, cap, and store at 2-8 degrees Celsius in an area designated for radiochemicals. Label the solution with nuclide, total activity, contact name, and date.

- C. Stock minerals and nutrients solution: create a 1:100 dilution of S3, S4, and S7 as presented by Owen et al. (1979) (all quantities reported in g/l).

1. S3: NH_4HPO_4 (26.7)
2. S4: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (16.7), NH_4Cl (26.6), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (120), KCl (86.7), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.33), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (2), H_3BO_3 (0.38), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.18), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.17), ZnCl_2 (0.14)

3. S7: biotin (0.002), folic acid (0.002), pyridoxine hydrochloride (0.01), riboflavin (0.005), thiamin (0.005), nicotinic acid (0.005), pantothenic acid (0.005), B₁₂ (0.0001), *p*-aminobenzoic acid (0.005), thiotic acid (0.005)
 - D. Imitation Vanilla: guaiacol (3.6), ethyl vanillin (1.2), propylene glycol (7.8), benzoate (0.8)
- II. Wash and rinse twelve 250 mL amber serum bottles. Place the caps into a clean empty beaker. Cover the bottles and beaker with aluminum foil and place them in the autoclave.
 - III. Turn on the purification furnace attached to the gas manifold system. Allow the furnace to reach operation temperature of 600 °C and regenerate copper catalyst using quick bursts of hydrogen gas.
 - IV. Use nitrogen gas that has passed through the purification furnace to maintain anaerobic conditions while extracting approximately 400 mL of anaerobic digester sludge from each source carboy. Label cells obtained from Corvallis Municipal Waste Water Treatment Plant on 9/26/95 as group "J" and cells obtained 2/27/97 as group "D". Deposit cells into two 500 mL Erlenmeyer flasks.
 - V. Cover the flasks with aluminum foil, mark the initial volume of cells in the flasks and place into the autoclave for sterilization. Add approximately 400 mL of D.I. water to a 500 mL beaker, cover with aluminum foil and place in autoclave.
 - VI. Add D.I. water to the autoclave (as needed), close the door, depress the reset button, set the exhaust to "slow", and turn the timer to sixty minutes. The autoclave needs to achieve a minimum temperature of 250 degrees F and pressure of 15 psig. Allow the autoclave to complete the run and cool. Repeat this step on two successive days to ensure complete sterilization.

VII. Remove the sterilized amber serum bottles, caps, cells, and water from the autoclave and place in the laminar flow hood. Use the sterilized water to return the sterile cell mass to the initial volume.

VIII. Prepare six bottles for each inocula (D and J). Label the active bottles as 1A, 1B, 2A, 2B and the controls as 3A and 3B. To each of the twelve bottles add the components listed below. After addition of inocula, the final volume in each bottle will be 100 mL.

- A. Nutrients (S3, S4, S7): 1 mL of dilute stock
- B. Imitation Vanilla Flavoring: 1 mL = 200 mg/L COD
- C. PCP Stock: 1.0 mL = 0.2 mg/L
- D. D.I. water: 68 mL

IX. Add 29 mL of sterile inocula to bottles D3A and D3B from source carboy "D". Cap and purge the headspace with purified nitrogen gas. Repeat for bottles J3A and J3B using sterilized cells from carboy "J".

X. Repeat steps three and four to acquire approximately 250 mL of viable cell mass from each anaerobic carboy. Maintain anaerobic conditions while adding 29 mL of viable inocula from carboy "D:" to bottles D1A, D1B, D2A, and D2B. Cap bottles using Teflon® coated septa and purge for several headspace volumes with purified nitrogen gas. Zero pressure in the bottles with a lubricated 10 mL glass ground syringe and conduct headspace analysis as possible to ensure no oxygen contamination has taken place. Repeat for bottles J1A, J1B, J2A, and J2B using appropriate inocula.

XI. Swirl bottles to mix and allow equilibration for approximately three hours. Conduct a chlorophenol analysis to determine the initial concentrations of chlorophenols in the bottles.

XII. Continue to monitor bottles with time until PCP has been completely dechlorinated to TCP.

- XIII. Respike all active bottles using PCP stock to achieve a final concentration of 0.2 mg PCP/L and continue to monitor bottles with time until PCP has once again been reduced to 3,4,5 TCP.
- XIV. Zero headspace pressure for all bottles and conduct headspace analysis. Completely purge headspace of each bottle with purified nitrogen gas.
- XV. Spike bottles D1B, D2B, D3B, J1B, J2B, and J3B with the required volume of PCP stock to achieve a final concentration of 0.5 mg/L. Store with radiolabelled bottles and Fisher recording thermometer in a secondary containment vessel under the hood in room 103. Continue to monitor bottles with time until PCP has been completely dechlorinated to 3,4,5 TCP. These bottles will serve as "mirror" studies to determine the appropriate time frame for conversion from anaerobic to aerobic processes for the radiolabelled bottles.
- XVI. Spike bottles D1A, D2A, D3A, J1A, J2A, and J3A with the required volume of UL-¹⁴C-PCP to achieve a final concentration of 0.5 mg/L UL-¹⁴C-PCP and an activity of 1.95 microcurries. Label each bottle with date, contact name, radionuclide, and "Caution Radioactive Material" tape. Store in a secondary containment vessel under the environmental health and safety approved hood in room 103
- XVII. Once sufficient degradation is observed in the mirror bottles "B", use 37 % HCl to decrease the pH to 6.0 and record volume of acid required. Add additional nutrients, vanilla, aerobic cells, and purge the headspace with oxygen. Continue to monitor the bottle headspaces for cumulative CO₂ production and perform chlorophenol assay every twenty-four hours.
- XVIII. Conduct liquid scintillation analysis for the headspace, supernatant, and solids of bottles "A". To avoid dropping below the desired pH and to avoid loss of activity due to volatilized labeled organic compounds, to each bottle "A" add 3/4 the total volume of HCl used to reduce the pH in the mirror bottles. Using the gas manifold and trap system, purge the reactor headspaces using compressed oxygen and trap effluent gases to check for volatile organic compounds.

Prepare samples according to the Liquid Scintillation Assay. Remove caps and add required volume of 37% HCl to reduce the pH to 6.0. Add vanilla, nutrients, aerobic inocula, and replace septa. Once again, purge the headspace of each bottle with oxygen, trap effluent gas and prepare samples to monitor for possible volatile organic compounds. After counting is complete, add BaCl₂ in excess to precipitate any CO₂ from scintillation cocktail and extract supernatant. Deposit supernatant in clean 20 mL vial with disposable polyethylene cap and place on Packard instrument for counting. Any counts above background can be attributed to volatile organic compounds. Monitor aerobic bottles for cumulative ¹⁴CO₂ production using the liquid scintillation assay as needed.

Appendix B

Liquid Scintillation Assay

Objective:

To use liquid scintillation analysis to verify complete mineralization of radiolabelled pentachlorophenol (UL- ^{14}C -PCP) to ^{14}C - CO_2 in a sequential anaerobic-aerobic biological batch reactor system.

Equipment:

Packard Tri-Carb Model 2500 TR/AB Liquid Scintillation Analyzer

Survey Meter: Ludlum III Geiger counter

Gas manifold and CO_2 traps

Compressed nitrogen cylinder and regulator

Compressed oxygen cylinder and regulator

Materials:

Reactors from anaerobic-aerobic sequential batch experiment.

Secondary containment vessel for storage of batch reactors

Environmental Health & Safety approved fume hood

1 x 10 μL metal syringe

1 x 100 μL metal syringe

1 x 1 mL Luer lock glass ground syringe

1 x 10mL Luer lock glass ground syringe with 22 gage needle

1 x 1 gallon reagent bottle for radioactive scintillation solution disposal

1 x 1L Nalgene™ polypropylene bottle for non-radioactive scintillation disposal

1 solid waste disposal container for dry radioactive wastes

20 mL scintillation vials with disposable polyethylene caps

1 Brinkmann Dispensette™ Bottle-Top Dispenser (5-25 mL)

Nitrile gloves

Chemicals:

Packard Permafluor® E⁺ liquid scintillation cocktail

Fisher ScintiSafe™ Econo 2 liquid scintillation cocktail

Parckard Carbo-Sorb® E CO₂ trapping agent

Deionized Water (D.I.)

Procedure:

I. For reactor preparation see, “Experimental Protocol for Sequential Anaerobic-Aerobic Batch Reactors.”

II. Sample Preparation: Use the Brinkmann Dispensette™ Bottle-Top Dispenser to pipette 10 mL of Fisher ScintiSafe™ Econo 2 liquid scintillation cocktail into 20 mL scintillation vials. Inject the following samples below the cocktail surface. Cap and place on Packard Tri-Carb Model 2500 TR/AB Liquid Scintillation Analyzer for counting.

- A. Supernatant: Use 100 μL metal syringe to extract 50 μL of supernatant from each reactor.
- B. Solids: Shake reactors until thoroughly mixed. Use the 10 μL metal syringe to extract a 10 μL aqueous sample.
- C. Headspace analysis: Use 10mL Luer lock glass ground syringe with 22 gage needle to extract a 2.5 mL sample from each reactor headspace.

III. Analysis of radiolabelled samples prepped during step 3 by Liquid Scintillation:

(Adapted from RUA 243-B, Pete Nelson / Mohammed Azizian for the Analysis of ^{14}C using LSC).

- A. Calibrate the liquid scintillation analyzer.
- B. Define the LSC protocol:
- C. Access status window and press the *edit protocol* function key
- D. Number and name the protocol
- E. Press the *count conditions* key and set the following:
- F. Count time = 60-120 minutes
- G. Cycles = 1
- H. Radionuclide = ^{14}C
- I. Count termination = use existing (set 2Sigma% = 2 for region A and B)
- J. Background Subtract = 1 vial
- K. Quench Indicator = tSIE
- L. Half-life correction = NO
- M. Special conditions = use exist

IV. *Loading and Running Samples: (taken from RUA 243-B)*

- A. Go to the status window and press the vials function key.
- B. Enter the protocol number.
- C. Load the samples into the cassettes in desired order. Reset protocol plug.
- D. Place the cassette on the right side of the sample exchange deck. Press the F-11 function key to start the count.

V. *Disposal:* Disposal of generated wastes will be conducted as specified in the RUA225-A Amendment and in accordance with the *OSU Radiation Safety Manual*.

VI. Continue to monitor non-radiolabelled parallel bottle study for conversion of PCP to TCP. After satisfactory conversion is observed, convert anaerobic labeled bottles to aerobic according to the Experimental Protocol for Sequential Anaerobic-Aerobic Batch Reactors.

Repeat step two substituting oxygen gas for nitrogen gas. Repeat step 4 multiple times to obtain a $^{14}\text{CO}_2$ production curve with time for each reactor.

Appendix C

Permanent Gas Assay

Objective:

Use the gas control system and a gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) to develop standard curves for batch reactor headspace analysis.

Materials / Equipment:

1*100 μ L gas-tight syringe

Gas sampling flask with rubber septa

HP-5890 series II GC equipped with a TCD

CarboxenTM 1000 60/80 stainless steel packed column; 15 ft

Cylinders of GC grade Argon, N₂, H₂, CH₄, CO₂, and O₂

Gas control system

Chemstation software package, HP 3365

J&W Scientific ADM 1000 Intelligent Flowmeter

Procedure:

- I. Prepare Chem Station: Load Method "HP GC TCD-DAVE.MTH"
- II. Program GC /TCD:
 - A. Injection temp: 275 °C
 - B. Detector temp: 275 °C
 - C. Carrier gas flow-rate (Argon): 35 mL / min
 - D. Initial oven temp: 35 °C

- E. Initial time: 5 min
 - F. Rate: 20 °C/min
 - G. Final oven temp: 225 °C
 - H. Final time: 5.5 min
- III. Prepare gas mixtures of known composition using the gas control system, gas cylinders, and gas flow meter. Follow operating instructions for gas controller system as written by Teresa Lemmon and Jim Ingle 08/20/92.
- IV. Use the gas sampling flask to contain gas of known composition while transporting to the GC for manual injection .
- V. Allow the GC to equilibrate. Use the 100 μ L syringe to extract a sample from the gas sampling flask . Inject the sample into the GC and simultaneously depress START. The chemstation will automatically begin recording data.

Appendix D

Chlorophenol Assay & Standard Curve Development

Objective:

This protocol is used for analyzing pentachlorophenol and its anaerobic metabolites on a gas chromatograph (GC) equipped with an electron capture detector (ECD). It has been adapted from Kaslik (1995) and was developed by Voss et al (1981). The method was later modified by Perkins et al. (1992) and miniaturized by Smith (1993).

Materials / Equipment:

2 * 100 μ L metal syringe

HP-6890 series II GC equipped with a TCD and a J&W Scientific DB-5MS 30m column

Chemstation software: Rev.A.05.01 [273], Copyright© Hewlett Packard, 1990-1997

1 x 1 mL fixed volume pipette

1 x 500 μ L repeating pipette

1 x 100 μ L repeating pipette

10 mL disposable culture tubes with Teflon[®] lined caps

2 mL capacity Borosilicate amber glass crimp top vials

12 mm crimp caps with Teflon[®]-Silicone septa

2 x 500 mL beakers (D.I. rinse and waste water)

1 x 10 mL beaker (hexane)

1 x 50 mL beaker (methanol rinse)

Disposable Pasteur pipettes and bulbs

Hand crimper

Wrist action shaker

Chemicals:

Acetic anhydride, reagent grade

Hexane, HPLC grade

Internal standard reagent (30.4 g/L K_2CO_3 , 500 mg/L 2,4,6 Tribromophenol)

Custom Standards: Ultra Scientific (North Kingstown, RI) Lot # J-2157

Procedure:

- I. Label each test tube according to sample contents and date
- II. To each test tube add the following:
 - A. 500 μ L of internal standard reagent
 - B. 100 μ L sample
 - C. 100 μ L acetic anhydride
- III. Prepare a method blank for each sample set with only A. and C. from step II.
- IV. Gently shake test tubes, place on wrist shaker, and set timer for twenty minutes.
- V. Remove test tubes from wrist shaker, place in test tube rack, and remove cap. As seal is broken a small release of gas should occur which verifies addition of acetic anhydride.
- VI. Add 1 mL of hexane to each test tube, cap, place on wrist shaker, and set timer for twenty minutes.
- VII. Remove test tubes from shaker, place in test tube rack, and remove caps. Use disposable Pasteur pipettes to extract hexane sample from test tubes (upper layer of dual non-aqueous phase liquid) and place hexane sample into crimp top vials. Cover with crimp cap and use hand crimper to seal vial.

VIII. Place sample vials on the autosampler rack, establish sequence table, load appropriate method (CP_32, or CP_321), and start sequence.

IX. Loading Method CP_32 or CP_321) will establish the following parameters for the GC-6890:

- A. Temperature Program
 - 1. Initial Temp: 40 °C
 - 2. Initial Time: 1.00 min
 - 3. Rate 1: 25 °C/min
 - 4. Final Temp: 140 °C
 - 5. Final Time: 0.0 min
 - 6. Rate 2: 10.00 °C/min
 - 7. Final Temp 2: 250 °C
 - 8. Final Time 2: 5.00 min
- B. Injection Temperature: 250 °C
- C. Detector Temperature: 350 °C
- D. Helium Program:
 - 1. Initial flow: 2.00 mL/min
 - 2. Initial Time: 14.00 min
 - 3. Rate 1: 4.00
 - 4. Final Flow: 4.00
 - 5. Final Time 7.00 min
- E. Argon Methane Program: 60 mL/min

Appendix E

List of Filenames

Microsoft Word *version 6.0* document

Thesis.doc Complete Thesis

Microsoft Excel *version 7.0* spreadsheets

BS6.xls Aerobic batch bioassay for removal of 3,4,5-TCP, start date (5-8-97)
BSJ1-3.xls Sequential anaerobic - aerobic batch bioassay, start date (5-21-97)
BSJ4-6.xls Sequential anaerobic - aerobic batch bioassay, start date (6-23-97)

Appendix F

Pertinent Figures Not Included in Chapter 2

Figures F.1 and F.2 were generated from data collected while conducting a second mineralization study and are analogous to Figures 2.3 and 2.4 of chapter 2. Figure F.3 was generated from data collected during the first mineralization study and is a duplicate for data presented in Figure 2.4 of Chapter 2.

Figure F.1 Similar PCP Removal Rates Demonstrated in Anaerobic Batch Bioassays (Bottle Study 2)

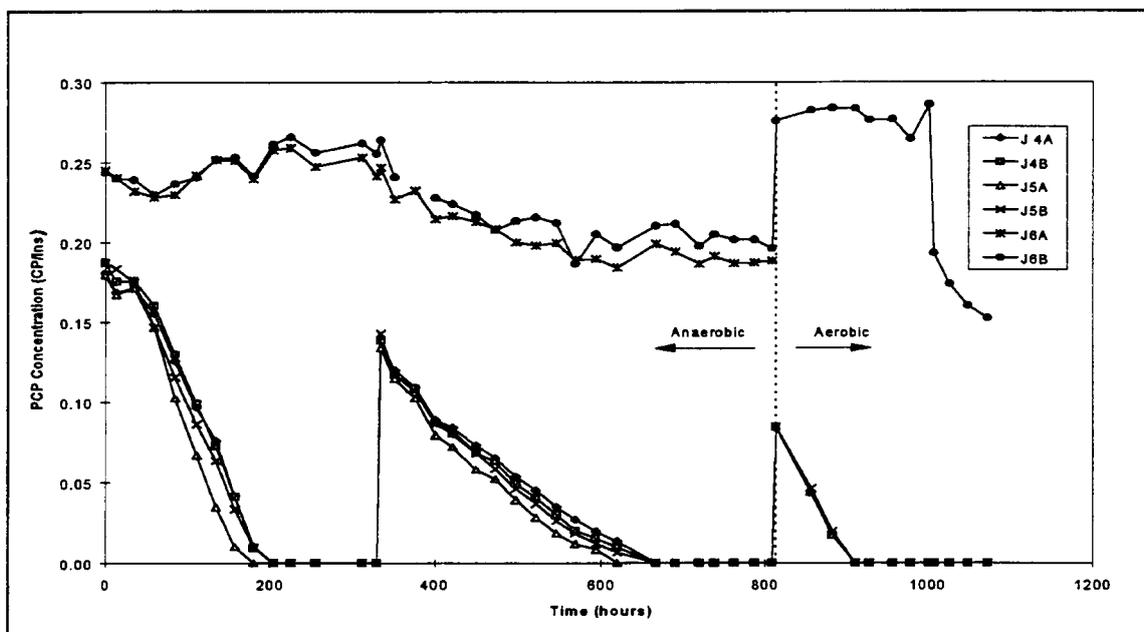


Figure F.2 Evidence for Mineralization in Labeled and Non-labeled Batch Bioassays (Bottle Study 2)

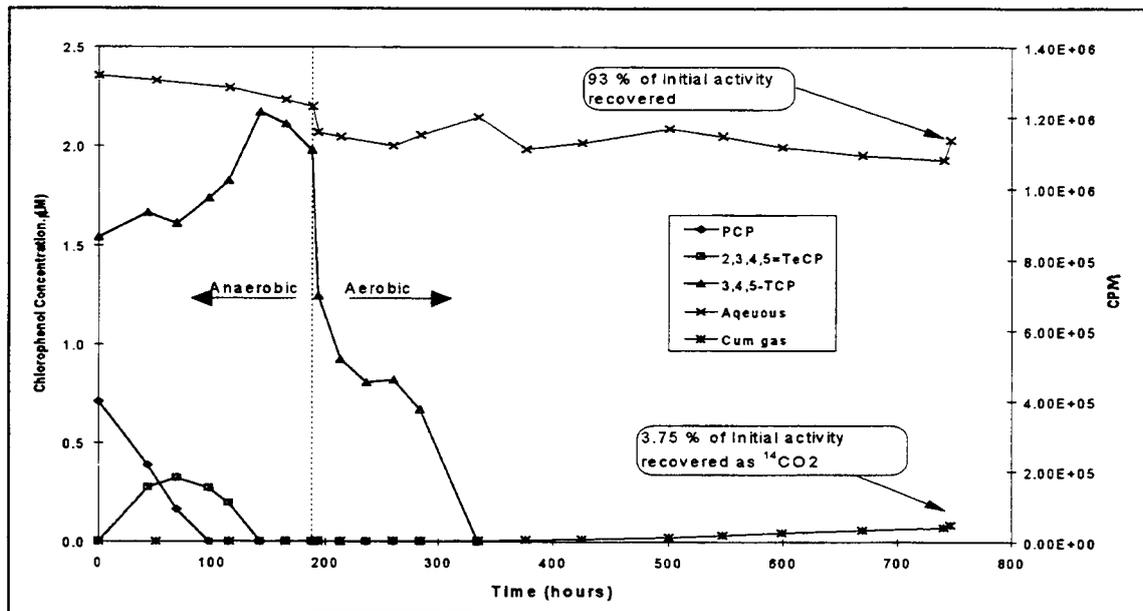
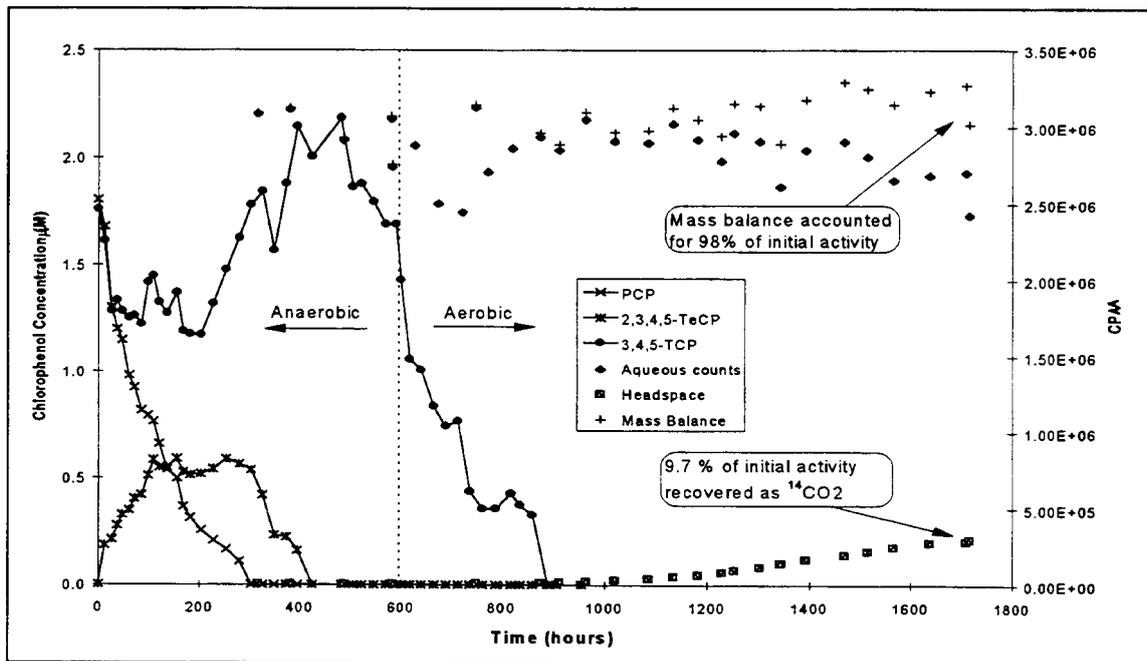


Figure F.3 Evidence for Mineralization in Labeled and Non-labeled Batch Bioassays (Bottle Study 1)



Appendix G

Solids Analysis for Batch Bioassays

The following solids were determined using Standard Methods 2540B and 2540E from Standard Methods for the examination of water and wastewater , 18th ed. (1992).

Table 4: Solids Analysis for Anaerobic Inoculum (5-21-97)

Sample No.	Volume (L)	Tare (g)	Tare + Solids (g)	Tare + Non-VS (g)	TS (g/L)	TVS (g/L)
1	0.015	15.3792	15.7665	15.6086	24.62	15.61
2	0.015	14.2242	14.5896	14.4328	24.36	14.43
3	0.015	14.3832	14.7437	14.2554	14.43	14.26
AVERAGE					24.34	10.44

Table 5: Solids Analysis for Aerobic Inoculum Conducted (5-21-97)

Sample No.	Volume (L)	Tare (g)	Tare + Solids (g)	Tare + Non-VS (g)	TS (g/L)	TVS (g/L)
1	0.010	18.2528	18.3764	18.3241	12.36	7.13
2	0.010	20.5045	20.6105	20.5672	10.60	6.27
3	0.010	17.6661	17.7717	17.7294	10.56	6.33
AVERAGE					11.20	6.60

Table 6: Solids Analysis for Bottle Study J1-J6 (8-28-97)

Sample No.	Volume in Bottle (L)	Tare (g)	Tare + Solids (g)	Tare + Non-VS (g)	TS (g/L)	TVS (g/L)
J1-1	0.151	1.0090	1.0364	1.0267	5.46	1.94
J1-2	0.151	1.0081	1.0329	1.0252	4.96	1.54
Average					5.22	1.74
J2-1	0.149	1.0077	1.0330	1.0242	5.08	1.76
J2-2	0.149	1.0148	1.0375	1.0324	5.40	1.88
Average					5.24	1.82
J3-1 (control)	0.134	0.9990	1.0277	1.0203	5.74	1.48
J3-2 (control)	0.134	1.0048	1.0331	1.0253	5.68	1.66
Average (control)					5.72	1.58
J4-1	0.155	1.0316	1.0316	1.0186	6.46	2.60
J4-2	0.155	1.0355	1.0355	1.0247	6.06	2.16
Average					6.26	2.38
J5-1	0.156	1.0318	1.0318	1.0236	5.28	1.64
J5-2	0.156	1.0110	1.0110	1.0084	3.28	0.52
Average					4.28	1.08
J6-1 (control)	0.134	1.0287	1.0287	1.0237	4.00	1.00
J6-2 (control)	0.134	1.0282	1.0282	1.0226	4.20	1.30
Average (control)					4.20	1.24

Sample volume was 0.005 L.

Appendix H

Groundwater Chemistry for Demonstration Site

Table 7: Groundwater General Chemistry for Demonstration Site (Remedial Technologies, Inc., 1994).

Sample Location		AGI-1	AGI-5N	AGI-7N	AGI-8	92-9
Date		10/90	10/90	4/93	4/93	4/93
PARAMETER (mg/L)	EPA Method					
<u>Inorganics</u>						
Calcium	7140	16	14		NA	NA
Chloride	325.5	< 5	< 5	NA	NA	NA
Iron	7380	22	24	0.058	< 5	< 5
Magnesium	7450	10	8	NA	NA	NA
Manganese	7460	NA	NA	0.01	0.14	1.2
Potassium	7610	1.5	1.2	NA	NA	NA
Sodium	7770	12	12	NA	NA	NA
Sulfate	9038	19.1	29.3	NA	NA	NA
Total Suspended Solids	160.2	1100	130	1600	< 10	< 10
Chemical Oxygen Demand	410.1	NA	NA	12	10.1	13.6
<u>Nutrients</u>						
Ammonia-Nitrogen	350.1	< 1	< 5	NA	NA	NA
Nitrate-Nitrogen	353.1	6.8	1.7	NA	NA	NA
Nitrite-Nitrogen	354.1	0.008	0.051	NA	NA	NA
Total Phosphate	365.4	0.75	0.53	NA	NA	NA