AN ABSTRACT OF THE DISSERTATION OF

Jason David Cole for the degree of Doctor of Philosophy in Civil Engineering presented on May 5, 2000.
Title: Demonstration of a Permeable Barrier Technology for the *In-situ* Bioremediation of Pentachlorophenol Contaminated Groundwater.

Abstract approved: Redacted for privacy

Sandra L. Woods

A pilot scale demonstration of a biological permeable barrier was conducted in a pentachlorophenol-contaminated aquifer at a wood preserving facility. A permeable reactor was constructed to fit within a large diameter well. Arranged in series, a cylindrical reactor 24'' x 36'' (0.61 x 0.91m) (diameter x height) was partitioned to provide three biological treatment zones. Pentachlorophenol (PCP) biodegradation was evaluated under several environmental conditions using a mixed microbial consortium supported on ceramic saddles. Imitation vanilla flavoring (IVF), a mixture of propylene glycol, guaiacol, ethyl vanillin and sodium benzoate, served as the electron donor. In the absence of exogenous substrate, PCP was not degraded in the inoculated permeable barrier. Substrate addition under oxidizing conditions also failed to initiate PCP removal. Anaerobic conditions however, promoted *in-situ* PCP degradation. PCP reductive dechlorination resulted in the transient production of 3,4,5-trichlorophenol through sequential *ortho* dechlorinations. Continued carbon reduction at the *meta* and *para* positions resulted in 3,4-dichlorophenol and 3,5-dichlorophenol production. Complete removal of all intermediate degradation products was observed. Reactor operation was characterized through two independent laboratory and field companion studies. Experiments were conducted to evaluate (1) the effect of supplemental electron donor concentration (IVF) and (2) the effect of sulfate, a competitive electron acceptor on PCP reductive dechlorination. Results from laboratory and field conditions were consistent. (1) In the presence of an exogenous electron donor, PCP degradation was independent of supplemental donor concentration (10, 25, 50, 100 mg COD/L). However, a comparatively slower rate of PCP degradation was observed in the absence of electron donor. (2) The presence of sulfate was not inhibitory to PCP degradation. However, compared to systems evaluated in the absence of sulfate, slower rates of PCP transformation were observed. Passive operation and low energy requirements, coupled with potential contaminant mineralization suggest that the biological permeable barrier is a highly effective tool for subsurface restoration.

Demonstration of a Permeable Barrier Technology for the *In-situ* Bioremediation of Pentachlorophenol Contaminated Groundwater

by

Jason David Cole

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APPROVED:

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Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Jason David Cole, Author

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DEDICATION

This dissertation is dedicated in loving memory of my grandmothers, Agnes Klim and Catherine Kowalczyk; they always wanted a doctor in the family.

PREFACE

This dissertation is written around the development of an *in-situ* biological treatment strategy for pentachlorophenol contaminated groundwater. The pilot scale technology was successfully demonstrated in a PCP-contaminated aquifer at the L. D. McFarland facility in Eugene, Oregon. Written in manuscript format, four individual technical papers in Chapter 2 through Chapter 5 summarize pertinent findings from field and laboratory research efforts. To maintain manuscript format, Chapter 6 is presented in the form of an individual paper. Publication however, is expected in the form of a technical note.

Demonstration of a Permeable Barrier Technology for the *In-situ* Bioremediation of Pentachlorophenol Contaminated Groundwater

CHAPTER 1

INTRODUCTION AND OBJECTIVES

In-situ bioremediation schemes often fail because a suitable substrate, the contaminant and viable microorganisms lack adequate mixing in the subsurface. Successful bioremediation projects often rely on pump and treat configurations with process reactors above ground. Although above ground reactors offer good process control, they suffer from high operation and maintenance costs. Efforts to increase the success of field based bioremediation systems have resulted in an array of technologies for subsurface treatment. Unfortunately, many of these technologies center on extraction and injection or infiltration methods to stimulate biological removal of the contaminant. In several cases, these technologies have been shown very effective for *in-situ* groundwater remediation (Dybas et al., 1998; Gersberg et al., 1995; Hooker et al., 1998; Hopkins and McCarty, 1995; Hopkins et al., 1993). Traditional treatment systems however posses inherent flaws: energy input for pumping the ground water, long term operation and maintenance costs, and the regulatory issues involved with reinjection limit their desirability for field scale bioremediation.

The benefits of groundwater remediation systems that minimize pumping are clear. Systems incorporating the use of subsurface recirculation show great potential for *in-situ* bioremediation. The system incorporates the use of a well screened over two intervals. The screened portions are hydraulically isolated from each other and water is pumped from one screened section and reinjected into the other. This technology minimizes pumping head required for water circulation and eliminates the regulatory issues associated with pumping ground water to the surface. Several applications of the dual screen recirculation system have been demonstrated using physical/chemical process for in-well contaminant removal. The biological applications of this technology have been limited (SBP, 1998). A recent full-scale demonstration by McCarty et al., 1998 incorporated the use of two recirculation wells for the aerobic cometabolic degradation of trichloroethylene (TCE) in the presence of toluene. The system performed very well, yet it was designed and constructed around unique geological conditions that may not be present on all sites.

The benefit of not removing water from the ground cannot be overemphasized when comparing remediation technologies. As such, recent attention has been directed toward the use of *in-situ* reactive walls or curtains. Conceptually derived by McMurty and Elton, 1985 and later expanded by Starr and

Cherry, 1994, the remediation system is based upon the interception of a contaminant plume down gradient from its source with a permeable yet reactive barrier. The barrier is placed within the aquifer structure so that groundwater is contacted and reacted with the media as it moves through the treatment wall. Contaminant free water exits on the down gradient side of the wall. Treatment of small plumes is accomplished using a single treatment wall. Whereas, interception and treatment of larger plume is achieved through a combination of multiple treatment walls and methods to control the subsurface groundwater flow.

Permeable barriers are applicable to a wide array of physical, chemical or biological treatment techniques. Operation of the system differs only by the reactive media chosen to construct the treatment wall. Construction media to support the adsorption of benzene from groundwater has been evaluated by Rael et al., 1995. While, reduction and precipitation of chromium(IV) to insoluble hydroxides of chromium(III) by iron bearing solids has been investigated by Blowes et al., 1997. Biological reactive walls have also been proposed for reduction of sulfate and precipitation of metals in leachate migrating from mine tailings (Waybrant et al., 1998). Current developments in reactive treatment media have focussed on zero valent metals and their ability for abiotic reduction of chlorinated solvents (Matheson and Tratnyek, 1994; Roberts et al., 1996). The use of zero valent iron permeable barriers for the reduction of chlorinated solvents at full-scale has been reported by Puls and Powell, 1997, including one case for a waste mixture of chromium(IV) and TCE.

In an effort to improve contaminant removal, minimize cost and maximize process control, a biological permeable reactor was developed for the treatment of pentachlorophenol (PCP)-contaminated groundwater. *In-situ* treatment is achieved using a large diameter well and a permeable biological reactor installed within the casing over screened interval of the contaminated aquifer. The reactor assembly is equipped with nutrient delivery and mixing systems for the support of a subsurface biological population. Reactor environmental conditions are controlled from the surface and allow the operation of three unique (e.g. anaerobic, aerobic) biological treatment zones (A, B and C). Biodegradation of the aqueous phase organic compounds occurs over the length of the reactor. Conceptual operation of the biological permeable barrier reactor for PCP degradation in a sequential anaerobic/aerobic environment is shown in Figure 1.1.

PCP was selected as a model compound for the demonstration project for many reasons. The compound is persistent in soil and groundwater; distribution and environmental release are widespread and biological degradation mechanisms share similar characteristics with many other highly chlorinated synthetic organic compounds. The United States Environmental Protection Agency (EPA) has classified PCP as a priority organic pollutant and imposed a Maximum Contaminant Level (MCL) value of 1μg/L in groundwater (Keith and Telliard, 1979). The human toxicity of PCP is largely unknown. The EPA, however, has ranked PCP as a class B2 carcinogen based on laboratory studies with mice (Institute, 1998). An active wood treating facility with PCP ground water contamination was chosen to demonstrate the

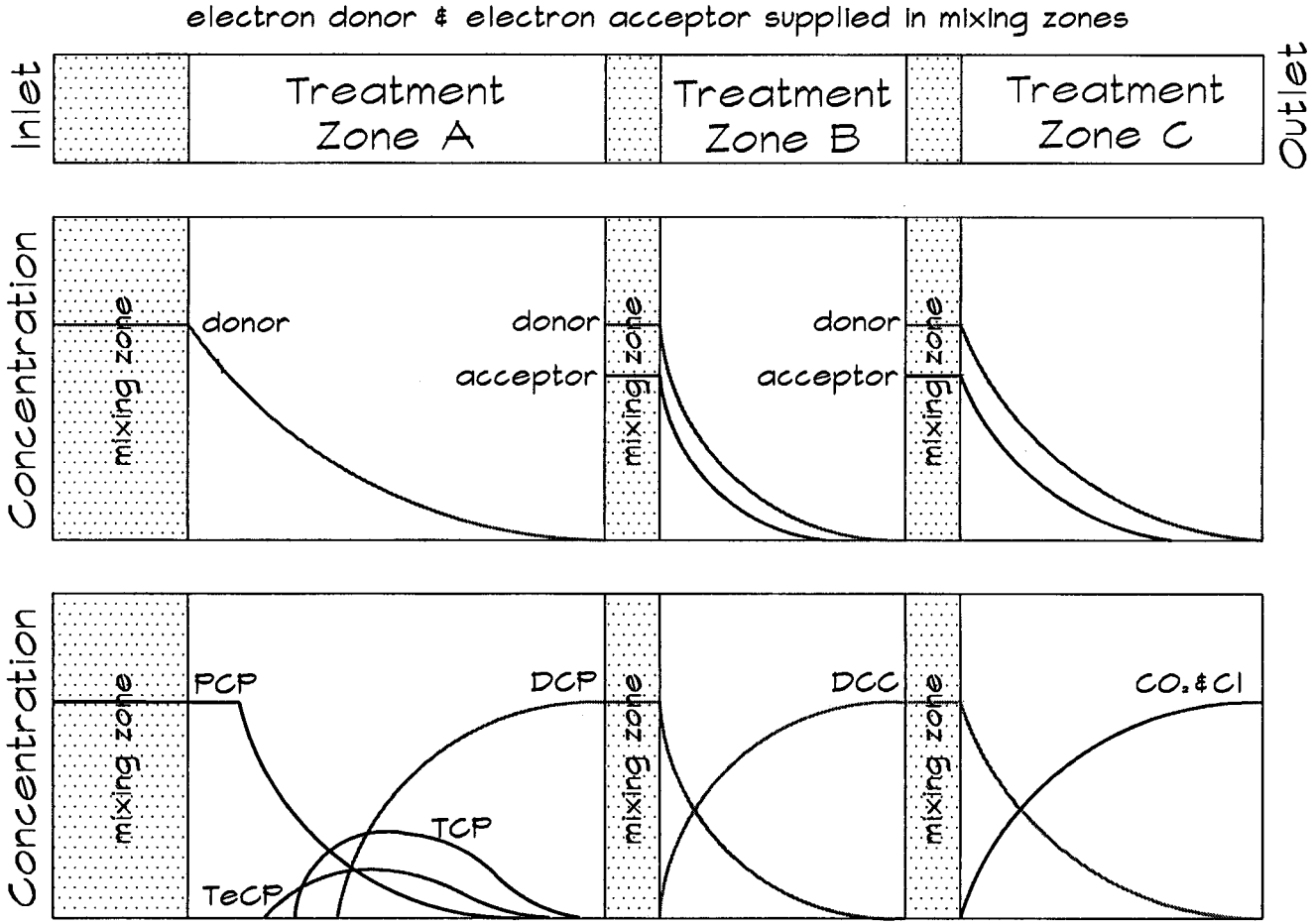

Figure 1.1 Conceptual treatment process for PCP contaminated groundwater

effectiveness of an *in-situ* permeable barrier treatment system operating under sequential anaerobic/aerobic treatment zones. PCP is primarily anthropogenic and has been commercially synthesized since the 1930s (Institute, 1998). It is a broad-spectrum biocide that is predominately used for the preservation of wood timbers, poles and fence posts (Crosby et al., 1981). Widespread contamination of soil and groundwater has resulted from the extensive use of PCP in the wood products industry. It is estimated that over 500 locations commercially used PCP in treating operations (Cirelli, 1978). Production and release of PCP to the environment has also been observed in effluents from pulp and paper manufacturing processes (Juteau et al., 1995b). On a much smaller scale, PCP has also been used for slime control in cooling towers, and as a fungicide in adhesives, paint, textiles and construction materials (Guthrie et al., 1984).

Degradation mechanisms of PCP and associated chlorinated phenolic compounds are well understood and have been evaluated under both aerobic and anaerobic conditions (Häggblom, 1990; Häggblom, 1992; Mohn and Tiedje, 1992). Under anaerobic conditions, reductive dechlorination processes result in chlorine removal and replacement with hydrogen on the aromatic ring (Boyd et al., 1983; Murthy et al., 1979). Reductive dechlorination of PCP has been observed at locations *ortho*, *meta* and *para* to the directing hydroxyl group. Anaerobic reductive dechlorination of chlorophenols has been observed in unacclimated and acclimated sewage sludge (Boyd and Shelton, 1984; Mikesell and Boyd, 1985; Mikesell and Boyd, 1986). Acclimation of the mixed microbial consortium to chlorophenols was found to influence the regiospecificty of dechlorination and overall degradation rates (Boyd and Shelton, 1984). Under anaerobic conditions, reductive dechlorination of PCP is favored in the presence of a suitable electron donor. PCP reductive dechlorination has been observed in the presence of a variety of exogenous electron donors (Madsen and Aamand, 1991; Woods et al., 1989; Nicholson et al., 1992; Jin and Bhattacharya, 1996; Duff et al., 1995). Yet, Boyd and Shelton, 1984; Boyd et al., 1983; Fathepure et al., 1988; Mikesell and Boyd, 1985; Mikesell and Boyd, 1986; Mikesell and Boyd, 1988 all observed PCP reductive dechlorination in the absence of an extrinsic electron donor. However, the addition of an external electron donor has been shown to enhance PCP reductive dechlorination by anaerobic consortiums.

RESEARCH PROGRAM ORGANIZATION

The organization and rationale for research presented in this dissertation follows the demonstration project flow chart presented in Figure 1.2. This project commenced with the **Process Development** for biological PCP degradation. Reductive dechlorination has been shown effective for the treatment of media impacted with chlorinated organic compounds. In an effort to maximize PCP transformation rates, degradation of PCP was evaluated under anaerobic conditions. Several studies were conducted in the laboratory to identify a suitable electron donor for anaerobic PCP reductive dechlorination. The results of the anaerobic electron donor study are presented in Chapter 2. The degradation of chlorophenols lacking full chlorine substitution was studied under aerobic environments. Electron acceptors for aerobic

degradation of 3,4-dichlorophenol and 3.5-dichlorophenol were evaluated (Kaslik, 1996). Results of the electron donor and acceptor studies supported the use of sequential anaerobic/aerobic treatment regimes for the rapid PCP transformation. To validate the treatment process, PCP degradation was evaluated under sequential anaerobic/aerobic environments. In the laboratory, C^{14}-labeled PCP was effectively mineralized to C^{14}-labeled carbon dioxide (Roberts, 1997).

Results of the laboratory studies supported the conceptual treatment regime and steps toward **Technology Development** were taken. A comprehensive subsurface characterization of the L.D. McFarland site in Eugene, Oregon was completed in 1993 (RETEC, 1994). Through subsurface boring and well construction logs, the location for the demonstration project was selected. The technology was slated for demonstration at the pilot scale. Therefore, it was decided to construct a small section of a permeable biological barrier in the aquifer. After evaluation of the subsurface stratum, construction methods were reviewed and costs were estimated for the site work. Installation of a rectangular section of a biological barrier was desired. However, construction of this type of configuration would have required the use of sheet pile supported excavation. The method was deemed to costly for the project scope and alternate methods were explored. Access to the subsurface contamination was eventually gained through a large diameter well. On site, a 24" diameter well was constructed for the demonstration project at considerable cost savings over traditional excavation techniques.

Technology development centered on a removable reactor system, which was designed to fit within the casing of the large diameter well. The reactor system was designed and constructed with three biological treatment zones separated by modular partitions. Between each treatment zone, provisions were made for the nutrient injection and mixing. Ceramic saddles were used in the biological treatment zones to provide surface for biological growth. The system was conceptually designed to operate under sequential anaerobic/aerobic environments. However, independent control of the mixing and nutrient addition within each treatment zone allowed for operational flexibility (e.g. complete aerobic or anaerobic operation). A comprehensive sample system was designed and installed into the reactor, which allowed for the collection of 28 discrete small volume aqueous samples. Placement of sample points with height, length and width allowed the complete characterization of PCP degradation with reactor space. To evaluate environmental conditions in the reactor treatment zones a system to continuously monitor the apparent oxidation/reduction potential and pH was designed. Once the permeable biological reactor was constructed, the designs for static unit support and installation were finalized. Material procurement and fabrication followed.

Preparation for the **Pilot Demonstration** began immediately following the construction of the large diameter well at the L.D. McFarland facility in Eugene, Oregon. Construction of a control system for the demonstration was not economically feasible. Therefore, the natural response of the aquifer in the absence of treatment was paramount in discerning the overall effectiveness of the treatment system. To ensure that changes in chlorophenol groundwater concentrations were a result of the permeable barrier installation, a

weekly monitoring program was started. Over a nine-month period prior to reactor installation, groundwater in the demonstration well was analyzed for chlorophenols and major anions. During the period of intensive baseline monitoring, the site was prepared for the system installation. A mobile field laboratory was setup to house the nutrient supply and sample systems. Final approvals were obtained from the facility and the Department of Environmental Quality. Once the operation of the ancillary support and sampling systems were installed and validated, conservative tracer studies were conducted to access the hydraulic residence time. Shortly afterwards the formal demonstration commenced when the reactor unit was inoculated with biomass.

The Technology Development, Process Development and Pilot Demonstration came together upon the installation of the biological permeable barrier. Operation of the biological permeable barrier followed an incremental procedure whereby chlorophenol response to physical changes evaluated (e.g. electron donor addition, mixing, etc.). Active PCP degradation in the treatment system was observed approximately three months after the installation of the biological permeable barrier. Results of the pilot demonstration for PCP degradation are presented in Chapter 3. Once active degradation of PCP was observed in the system, companion laboratory and field experiments were conducted. These experiments commenced with a study tailored to evaluate the effects of electron donor concentration on PCP reductive dechlorination. Results from the field and companion laboratory based experiments are presented in Chapter 4. To evaluate the potential for the application of biologically mediated reductive dechlorination in the presence of sulfate a second companion study was undertaken. In the laboratory and under field conditions, PCP reductive dechlorination was evaluated in the presence of sulfate, a competitive electron acceptor. Pertinent findings of the sulfate companion study are presented in Chapter 5.

Under all field conditions evaluated, complete PCP degradation was observed in the biological permeable barrier. However, companion laboratory based studies were unable to remove PCP completely and 3,4,5-trichlorophenol (3,4,5-TCP), an intermediate degradation product accumulated. Sensing the product accumulation was perhaps a result of hydrogen limiting conditions in serum bottles, additional laboratory studies were conducted. Using organisms harvested from the L.D. McFarland site, the degradation of 3,4,5-TCP as a function of hydrogen partial pressure was evaluated. Implications of the study on the development of biological treatment strategies for PCP contaminated groundwater are presented in Chapter 6.

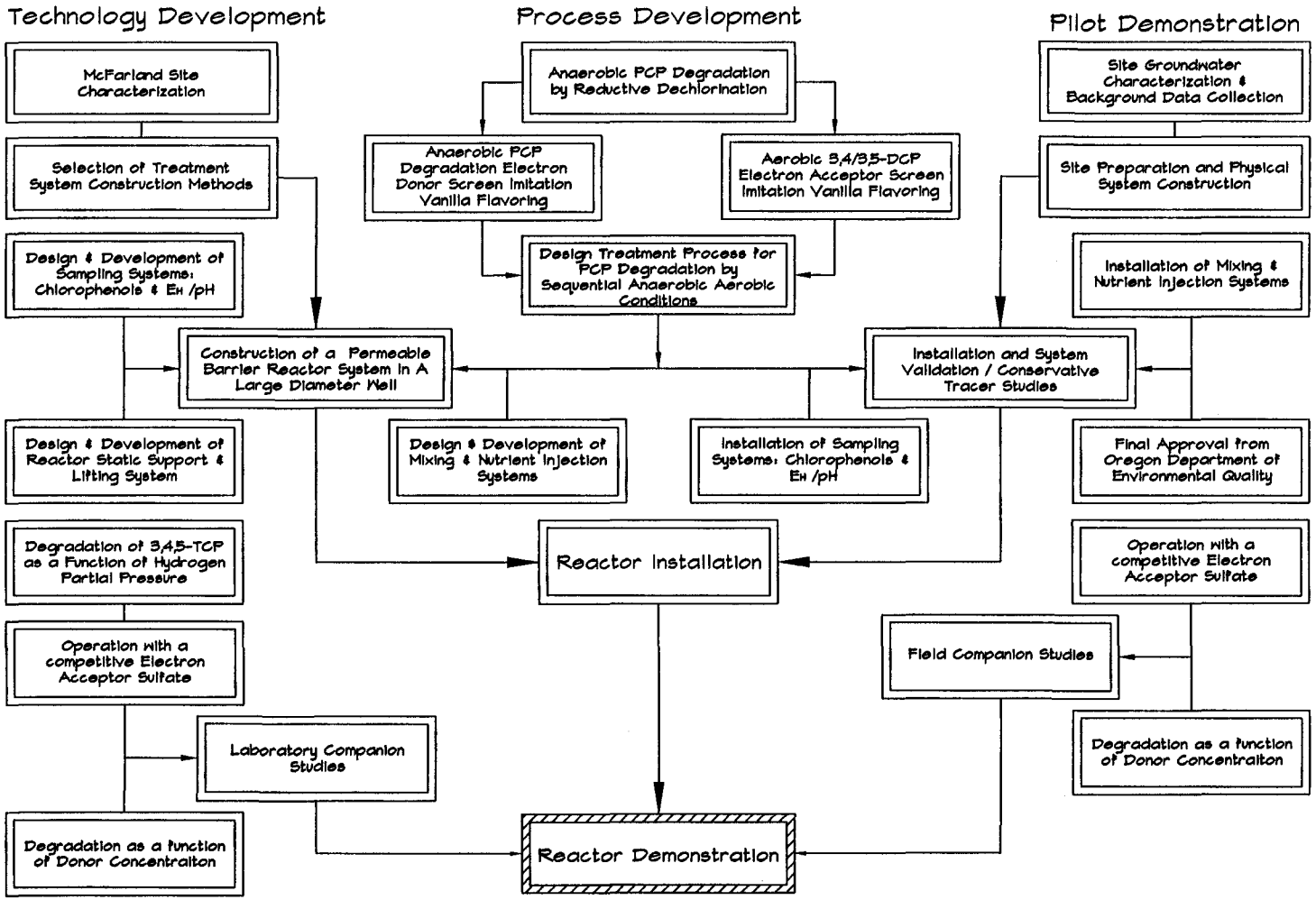

Figure 1.2 Demonstration Project Flow Chart

RESEARCH PROGRAM GOAL

Develop a biological permeable barrier technology for the in-situ treatment of pentachlorophenol-contaminated groundwater.

In fulfillment of the program goal, research and development of the treatment system were distributed among three areas of focus: Process Development, Technology Development and the Pilot Demonstration. In an effort to meet the goal of the research program a variety of tasks were undertaken. As previously described these tasks loosely followed the project flow chart depicted in Figure 1.2. Specific research goals of the individual project phases are listed below and summarized by chapter.

Chapter 2 Evaluation of Imitation Vanilla Flavoring to Support the Reductive Dechlorination of Pentachlorophenol

In the development of an *in-situ* treatment technology for the bioremediation of pentachlorophenol-contaminated groundwater, the need for an electron donor to support an anaerobic consortium was identified. Imitation vanilla flavoring showed potential in its ability to support a biological population capable of PCP reductive dechlorination. All of the chemical components of the imitation vanilla flavoring are "Generally Recognized as Safe" (GRAS) by the U.S. Food and Drug Administration. Therefore, it was believed that the use of imitation vanilla flavoring in a field based remediation scheme would be acceptable to the site owners and regulatory community. In an effort to determine the applicability of imitation vanilla flavoring for use in a PCP bioremediation scheme, this study was undertaken setting forth the following goals:

- To investigate the feasibility of imitation vanilla flavoring to support the growth of an anaerobic PCP degrading culture.
- To verify biological PCP transformation when of imitation vanilla flavoring serves as an electron donor.
- To characterize the pathway of PCP dechlorination when imitation vanilla flavoring serves as an electron donor.
- To evaluate the removal efficiency and rate of PCP dechlorination when imitation vanilla flavoring serves as an electron donor.

Chapter 3 Pilot Scale Demonstration of a Permeable Barrier Technology for the *in-situ* Bioremediation of Pentachlorophenol-Contaminated Ground Water

Traditional permeable reactive barriers employing degradation processes catalyzed by zero valent metals are ineffective for the remediation of materials impacted by chlorinated aromatic compounds. The focus of this research was to design and demonstrate a permeable barrier for the biological degradation of highly chlorinated aromatic compounds. The use of biological treatment process rather than abiotic

reduction is a significant deviation in current permeable barrier research and field applications. Through this deviation, it was hoped to expand the realm of contaminants applicable to permeable barrier treatment strategies. Having previously identified a conceptual biological treatment regime for pentachlorophenol (PCP) impacted groundwater, the design of a biological permeable barrier was undertaken. For many reasons, PCP was a desirable demonstration compound. It is chemically stable, persistent in soil and groundwater, widely distributed in the environment and biological degradation mechanisms are similar to other halogenated synthetic organic compounds. In an effort to develop a passive *in-situ* biological treatment strategy for PCP-contaminated groundwater, this study was undertaken setting forth the following goals:

Develop a passive bioremediation system for ground water interception and design a functional, cost effective experimental system to conduct in-situ PCP biodegradation studies.

- Assess the suitability of location for a pilot scale demonstration.
- Evaluate and characterize the subsurface conditions present at the L.D. McFarland facility in Eugene, Oregon.
- Evaluate the effects of seasonal water table variations as a function of chlorophenol congener and concentration.
- Determine the mean hydraulic residence time for the system using a conservative tracer method.

Design a removable permeable barrier reactor with the capacity for process sample collection, mixing, nutrient injection, and ample surface area for cellular growth.

- Using the physical constraints imposed by the demonstration location and the results of previous degradation studies, design the process for the biological degradation of PCP.
- Design the structural components of the reactor housing that will support the packing material during installation and operational conditions.
- Fabricate the reactor with materials able to withstand the rigors of operation in a chemically harsh environment.
- Design, install and validate a system to provide adequate mixing of the electron donors, acceptors and microorganisms without disrupting groundwater flow fields.
- Design, install and validate a sample system capable of small volume collection within the reactor assembly.
- Design, install and validate a system capable of real time data collection to monitor environmental conditions present within the permeable barrier reactor assembly (e.g. pH, oxidation/reduction potential, conductivity, etc.).
- Install the reactor assembly without packing material to evaluate ancillary system operation.

Demonstrate the in-situ removal of PCP, a model compound in a biological permeable barrier reactor.

- To evaluate the removal of PCP in the absence of inoculum.
- Evaluate the performance of the permeable reactor system in the presence and absence of electron donor supply.
- Evaluate PCP degradation under anaerobic and sequential anaerobic/aerobic environments.

Chapter 4 Field and Laboratory Comparisons of Substrate Requirements for the Bioremediation of Pentachlorophenol-Contaminated Ground Water

In studies of anaerobic pentachlorophenol (PCP) reductive dechlorination, toxicity of the target compound, PCP is often of more concern than the concentration of supplemental electron donor. While PCP toxicity to the anaerobic culture is of great importance to the success of the biological remediation strategy, the contribution and potential effects of supplemental electron donor addition cannot be casually overlooked. To better understand the relationship between electron donor concentration and reductive dechlorination, PCP degradation was evaluated as a function of supplemental electron donor concentration. Parallel degradation studies were conducted in the field and laboratory. Field based experiments were conducted in a pilot scale biological permeable barrier reactor. The reactor was fabricated to fit within the casing of a large diameter well that was constructed in a PCP-contaminated aquifer. Laboratory studies were conducted in batch serum bottles. Specifically, this comparison study was undertaken with the following objectives:

- Determine the effect of electron donor concentration on the reductive dechlorination of PCP under field and laboratory conditions.
- Evaluate the pathway of PCP reductive dechlorination under field and laboratory conditions.
- Investigate the potential for the use of alternate electron donors in the pilot scale permeable barrier reactor.
- Optimize operation of the pilot scale reactor through the identification of threshold electron donor concentrations needed to support PCP reductive dechlorination.

Chapter 5 The Effect of Sulfate on the Reductive Dechlorination of Pentachlorophenol: A Field and Laboratory Comparison

Reductive dechlorination is a biologically catalyzed oxidation/reduction reaction where the chlorinated compound, acting as an electron acceptor is reduced. Like all redox reactions, electron flow is generated through the oxidation of an electron donor. In anaerobic environments where reductive dechlorination is favored, terminal electron acceptors like sulfate compete for available reductant. In the application of an anaerobic treatment regime for chlorinated groundwater contaminants, the effectiveness of reductive dechlorination may be compromised by a microbial population competing for available donor for sulfate reduction. Therefore, this study was designed to estimate the feasibility of biological treatment strategies for chlorinated compounds in groundwater systems containing sulfate. Anaerobic pentachlorophenol

degradation in the presence of a competitive electron acceptor, sulfate, was evaluated under laboratory and field conditions. Field based experiments were conducted at the pilot scale using a custom designed permeable barrier reactor. The reactor assembly was fabricated to fit within the casing of a large diameter well that was constructed in a PCP-contaminated aquifer at the L.D. McFarland facility in Eugene, Oregon. Specifically, this field and laboratory comparison study was undertaken with the following objectives:

- Determine the effect of sulfate on the reductive dechlorination of PCP under field and laboratory conditions.
- Evaluate the pathway of PCP reductive dechlorination under field and laboratory conditions in the presence and absence of a competitive electron acceptor.
- Investigate the feasibility for anaerobic biological treatment strategies for the remediation of groundwater containing chloroaromatics and sulfate.

Chapter 6 The Effects of Hydrogen on the Reductive Dechlorination of 3,4,5-Trichlorophenol

The accumulation of 3,4,5-TCP from PCP reductive dechlorination is a potential problem in the application of biological treatment techniques for PCP contaminated groundwater. Laboratory research suggested that 3,4,5-TCP accumulation might occur in systems that are hydrogen limited. With the following objectives, this study was undertaken to determine the effect of a hydrogen partial pressure on the reductive dechlorination of 3,4,5-trichlorophenol.

- Evaluate the potential of 3,4,5-TCP reductive dechlorination when hydrogen is supplied as an exogenous electron donor.
- To test the hypothesis that 3,4,5-TCP accumulation in previously studied serum bottles resulted from a lack of hydrogen.
- Estimate the hydrogen concentration requisite for active 3,4,5-TCP reductive dechlorination.
- Investigate the pathway of 3,4,5-TCP degradation when hydrogen acts the electron donor.
- Examine the potential for the use of hydrogen in a field based remediation scheme.

CHAPTER 2

EVALUATION OF IMITATION VANILLA FLAVORING TO SUPPORT THE REDUCTIVE DECHLORINATION OF PENTACHLOROPHENOL

Jason David Cole

To be submitted to Water Research
International Association on Water Quality, London, U.K.

ABSTRACT

In serum bottle assays, the degradation of pentachlorophenol (PCP) was evaluated using an acclimated anaerobic consortium maintained at 21±2°C. The consortium originated as a mixture of municipal wastewater sludge (50:50 v:v anaerobic digester supernatant and return activated sludge). Imitation vanilla flavoring, a mixture of propylene glycol, guaiacol, ethyl vanillin and sodium benzoate, served as the system electron donor. Duplicate serum bottles were inoculated with 800 mg VSS, 1 g carbonaceous oxygen demand (COD)/L imitation vanilla flavoring and 2.25μM PCP. Parallel controls, conducted in duplicate were heat sterilized to assess mechanisms of abiotic PCP removal. In the biologically active bottles, PCP was rapidly transformed by reductive dechlorination. While complete PCP removal was not observed in the study, approximately 99% of the initial mass was transformed within 85 hours. PCP was not appreciably removed in the control system. The reductive dechlorination of PCP was observed to proceed by two distinct pathways. Primary PCP degradation followed the initial cleavage of an *ortho* chlorine atom to yield 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP). Produced transiently, 2,3,4,5-TeCP yielded 3,4,5-trichlorophenol (3,4,5-TCP). With time 3,4,5-TCP concentrations increased. Following the 3,4,5-TCP production, increasing concentrations of 3,4-dichlorophenol (3,4-DCP) and 3,5-dichlorophenol (3,5-DCP) were observed. To a lesser extent the initial degradation of PCP was also catalyzed though the removal of a *para* substituted position which formed 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP). Immediate production of 2,3,5-trichlorophenol (2,3,5-TCP followed the observation of 2,3,5,6-TeCP indicating the removal of an *ortho* substituted chlorine atom. Imitation vanilla flavoring was an effective electron donor to support the anaerobic reductive dechlorination of PCP. Evaluation of the PCP degradation pathway indicates the ability of the consortium to remove chlorine atoms from the *meta*, *para*, and *ortho* substituted positions.

RESEARCH OBJECTIVES

In the development of an *in-situ* treatment technology for the bioremediation of pentachlorophenol-contaminated groundwater, the need for an electron donor to support an anaerobic consortium was identified. Imitation vanilla flavoring showed potential in its ability to support a biological population capable of PCP reductive dechlorination. All of the chemical components of the imitation vanilla flavoring are "Generally Recognized as Safe" (GRAS) by the U.S. Food and Drug Administration. Therefore, it was believed that the use of imitation vanilla flavoring in a field based remediation scheme would be acceptable to the site owners and regulatory community. In an effort to determine the applicability of imitation vanilla flavoring for use in a PCP bioremediation scheme, this study was undertaken setting forth the following goals:

- To investigate the feasibility of imitation vanilla flavoring to support the growth of an anaerobic PCP degrading culture.

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- To characterize the pathway of PCP dechlorination when imitation vanilla flavoring serves as an electron donor.
- To evaluate the removal efficiency and rate of PCP dechlorination when imitation vanilla flavoring serves as an electron donor.

INTRODUCTION

Pentachlorophenol is a broad-spectrum biocide that is predominately used for the preservation of wood timbers, poles and fence posts (Crosby et al., 1981). The extensive use of pentachlorophenol (PCP) in the wood preservation industry has resulted in widespread contamination of soil and groundwater. Cirelli, 1978 reported the commercial use of PCP at over 500 locations in the United States. Many of the sites listed under the National Priorities List are active or abandoned wood treatment facilities that once used PCP heavily in treatment operations. On a much smaller scale, PCP has also been used for slime control in cooling towers, and as a fungicide in adhesives, paint, textiles and construction materials (Guthrie et al., 1984). Ide et al., 1972 has reported the use of PCP as a herbicide in Asian rice paddy soils.

PCP is primarily anthropogenic and has been commercially synthesized since the 1930s (Institute, 1998). Production and release of PCP to the environment has also been observed in effluents from pulp and paper manufacturing processes (Juteau et al., 1995b). The United States Environmental Protection Agency (EPA) has classified PCP as a priority organic pollutant and imposed a Maximum Contaminant Level (MCL) value of 1μg/L in groundwater (Keith and Telliard, 1979). The human toxicity of PCP is largely unknown. The EPA, however, has ranked PCP as a class B2 carcinogen based on laboratory studies with mice (Institute, 1998). Concern over the toxicological effects of PCP contamination, in soil and ground water has spawned a wealth of investigations aimed to determine the compounds ultimate environmental fate.

Despite its biocidal nature, PCP degradation has been observed in natural and experimental systems under a diverse range of environmental conditions. Microbial degradation of aqueous phase PCP has been shown using both mixed and pure cultures. Brown et al., 1986 and Moos et al., 1983) observed the removal of PCP from wastewater using an aerobic consortium in laboratory scale reactors. Pure aerobic cultures from the bacterial strains *Flavobacterium* and *Rhodoccus* were shown to mineralize PCP in both a continuous flow stir tank rector (CFSTR) and batch experiments (Apajalahti and Salkinoja-Salonen, 1986; Brown et al., 1986). Valo et al., 1990 demonstrated the use of two strains of *Rhodoccus* immobilized on polyurethane beads for the aerobic mineralization of synthetic PCP-contaminated ground water in a plug flow reactor. Several strains of pure aerobic cultures have also been shown capable of PCP degradation in

soil (Edgehill, 1994; Pfender, 1996). Fungi of the genus *Phanerochate* also possess mechanisms to degrade PCP (Lamar et al., 1990; Mileski et al., 1988; Pfender et al., 1997).

The degradation of PCP under anaerobic conditions is well documented. Under anaerobic conditions, reductive dechlorination processes result in chlorine removal and replacement with hydrogen on the aromatic ring (Boyd et al., 1983; Murthy et al., 1979). Reductive dechlorination of PCP has been observed at locations *ortho*, *meta* and *para* to the directing hydroxyl group. Anaerobic reductive dechlorination of chlorophenols has been observed in unacclimated and acclimated sewage sludge (Boyd and Shelton, 1984; Mikesell and Boyd, 1985; Mikesell and Boyd, 1986). Acclimation of the mixed microbial consortium to chlorophenols was found to influence the regiospecificty of dechlorination and overall degradation rates (Boyd and Shelton, 1984). Mikesell and Boyd, 1986; Nicholson et al., 1992; and Woods et al., 1989 determined that unacclimated sewage sludge preferentially degraded PCP by sequential *ortho* dechlorination to yield 3,4,5-trichlorophenol which accumulated. However, when the sludge had been acclimated to chlorophenols, PCP degradation to monochlorophenol occurred with dechlorination at all positions. In contrast to the previous observations, Bryant et al., 1991 observed with unacclimated anaerobic sediments that PCP was initially dechlorinated in the *para* position forming 2,3,5,6-tetrachlorophenol. Hendriksen et al., 1992 observed the initial *para* dechlorination of PCP in an upflow anaerobic sludge blanket (USAB) reactor inoculated with unacclimated granular sludge and amended with phenol and glucose. Dechlorination of PCP at the *meta* position by an unacclimated culture was observed in a fluidized-bed granular activated carbon reactor fed a continuous stream of PCP and ethanol (Khodadoust et al., 1997). It is clear from published results that factors other than acclimation can influence the position of dechlorination in anaerobic PCP degradation.

Dechlorination of PCP has been observed with several types of inoculum, electron donor and reactor configuration (Table 2.1). Many groups have shown the production and accumulation of intermediate products of PCP dechlorination (Boyd and Shelton, 1984; Cole, 1993; Madsen and Aamand, 1991). Metabolites of PCP vary in their degree of microbial toxicity (Ruckdeschel et al., 1987). Incubations of PCP and several common microbial intermediates were conducted with 30 strains of various bacterial species. In 26 of the 30 strains tested, 3,4,5-TCP exhibited the highest toxicity. Toxicity assays by Mikesell and Boyd, 1986 and Bryant et al., 1991 found 3,4,5-TCP 5 times more mutagenic than PCP. In anaerobic systems, 3,4,5-TCP concentrations greater than five mg/l have also been shown to inhibit methanogenesis (Woods, 1985). Serum bottle studies conducted by Liu et al., 1996 with anaerobic chlorophenol acclimated sediments showed transformation of 3,4,5-TCP as a rate limiting step in the transformation of PCP to 3,5-dichlorophenol. In acetate-fed, PCP acclimated cultures, sequential *ortho* dechlorination occurred producing 3,4,5-TCP, which accumulated (Cole et al., 1996). Similar degradation pathways were observed by Stuart and Woods, 1998 despite changes in the electron donor (methanol, acetate/methanol and hydrogen). In both cases, PCP degradation was incomplete and not apparently inhibited by the presence of 3,4,5-TCP in the reactors. The accumulation of intermediate metabolites,

especially those more toxic than the parent compounds, is a reoccurring problem in the application of biologically based treatment regimes (Zitomer and Speece, 1993). Successful application of biological treatment techniques therefore requires a thorough understanding of the target compound's degradation pathway.

The pathway by which dechlorination of PCP proceeds may have a profound effect on the overall success of microbial-based remediation scheme. Initial PCP dechlorination at the *para* position is desirable and would eliminate the potential for 3,4,5-TCP production. Furthermore, DeMarini et al., 1990 demonstrated that removal of PCPs *para* chlorine results in intermediate products less mutagenic than PCP. Accumulation of 3,4,5-TCP has been observed in both acclimated and unacclimated systems. The reasons for one system's ability to acclimate and dechlorinate PCP through 3,4,5-TCP and another system's lack thereof are unclear but, may depend on the hydrogen partial pressure or other nutrient limiting conditions. Cases presented in Table 2.1 show no clear pattern between culture acclimation or initial source and the dechlorination pathway observed.

Many factors contribute to a culture's ability to reductively dechlorinate PCP. Environmental conditions, such as temperature, oxidation-reduction potential and nutritional requirements of the inoculum play an essential role in the potential biodegradation of a xenobiotic compound. Reductive dechlorination requires a reductant source. Easily degradable compounds (e.g., methanol, acetate, glucose) supplied to the anaerobic culture provide the necessary reducing power to make reductive dechlorination favorable. Table 2.1 provides a sample of the diverse range of electron donors capable of supporting reductive dechlorination. The presence or absence of a reductant source may be related to the initial position of PCP dechlorination. Figure 2.1 represents a comparison of initial dechlorination position as a function of electron donor supplied for PCP degradation studies summarized in Table 2.1. In most cases observed, aliphatic based carbon compounds served as the electron donor for the mixed cultures. However, several research groups selected aromatic compounds or a mixture of aliphatic and aromatic compounds as electron donors. The relative percentage of initial dechlorination location shown in Figure 2.1 was computed from the published results of 27 PCP degradation studies. Differences among the studies prevent direct comparison. However, the figure suggests a difference between the initial dechlorination position and electron donor supplied. Electron donors that were aromatic in nature resulted in nearly three times as many *para* dechlorinations than was observed in systems provided aliphatic donors.

Complete reduction of PCP or any other chlorinated phenol will ultimately yield the production of phenol (Bryant et al., 1991; Juteau et al., 1995a; Kennes et al., 1996; Mikesell and Boyd, 1986; Zhang and Wiegel, 1990). Production of phenol in the reductive system marks an important biological step in the mineralization of the chlorinated parent compound. Degradation studies by Zhang and Wiegel, 1990 found that low phenol concentrations decreased the lag time required for the removal of 4-chlorophenol. Phenol present in the system was degraded to acetate, carbon dioxide and hydrogen through benzoate. In studies

Table 2.1 Dechlorination pathway as a function of inoculum and electron donor

Inoculum[1]	E. Donor	Pathway	Method	Reference
Sludge-U		PCP→NR→3,4,5→3,5	S.B[5].	(Mikesell and Boyd, 1985)
Sludge-A		PCP→2,3,4,5→3,4,5→3,5→3 →CH_4/CO_2	S.B[5]	(Mikesell and Boyd, 1986)
Sludge-U Sludge-A	Acetate, methanol[2]	PCP→2,3,4,5→3,4,5 PCP→2,3,4,5→3,4,5→3,5→3	CFSTR[6]	(Woods et al., 1989)
Pond sed-U Pond sed-A		PCP→2,3,5,6→2,3,5 PCP→2,3,5,6→2,3,5→3,5→3→phenol	S.B[5] S.B[5]	(Bryant et al., 1991)
Manure-U Lake sed-U Stream sed-U Swamp sed-U	Phenol, ethanol[2]	PCP→2,3,4,5→3,4,5→3,5 PCP→2,3,5,6→2,3,5→3,5 PCP→2,3,4,5→3,4,5→3,5 PCP→2,3,5,6→2,3,5→3,5 PCP→2,3,4,5→3,4,5→3,5 PCP→2,3,5,6→2,3,5→3,5	S.B[5]	(Larsen et al., 1991)
Sludge-A	Hydrogen[4]	PCP→2,3,4,5→3,4,5→3,5→3	S.B[5]	(Madsen and Aamand, 1991)
Anaerobic sludge-U	Phenol, glucose[2]	PCP→2,3,5,6→2,3,5→3,5→3 (primary) PCP→2,3,4,5→3,4,5→(3,5/3,4)→3	UASB	(Hendriksen et al., 1992)
Sludge-U Sludge-A	Acetate	PCP→2,3,4,5→3,4,5→3,4 PCP→2,3,5,6→2,3,5→3,5 PCP→2,3,4,6→2,4,6→2,4 PCP→2,3,4,6→2,4,6→2,4 PCP→2,3,4,6→2,4,5→(2,4/3,4)	CSFTR[6] B.R.[5]	(Nicholson et al., 1992)
Anaerobic sludge-U	Phenol	PCP→2,3,5,6→2,3,5→3,5→3	UASB[7]	(Duff et al., 1995)
Sludge-A	Glucose, formate[2]	PCP→2,3,5,6→2,3,5→3,5→3→phenol	CFFFR[8]	(Juteau et al., 1995a)
Anaerobic sludge-U	Propionate	PCP→NR→2,4,6→2,4→4 (primary) PCP→NR→2,3,5→3,5→3	S.B[5]	(Jin & Bhattacharya, 1996)
Sludge & sed mixture-A	Acetate , butyrate, methanol, propionate[2]	PCP→NR→2,4,6→2,4→(2/4)→phenol →CH_4/CO_2 [10]	S.B[5]	(Kennes et al., 1996)
Estuarine sed-U & A		PCP→2,3,4,5→3,4,5→3,5→3	S.B[5]	(Liu et al., 1996)
Anaerobic WW-U	Ethanol	PCP→NR→2,4,6→2,4→4 (initial) PCP→NR→3,4,5→(3,4/3,5)→(3/4)	GAC-FBR[9]	(Khodadoust et al., 1997)
Pure culture	Glucose, formate[2]	PCP→NR→(3,4,5/2,3,5)→(3,5/3,4)	S.B[5]	(Beaudet et al., 1998)
Sludge-A	Acetate, lactate, pyruvate[2]	PCP→2,3,4,5→3,4,5→3,5→3	B.R.[5]	(Chang et al., 1998a)

Notes:

1. A-Chlorophenol acclimated; U-Unacclimated; Sed. – sediment; sludge-harvest from sewage
2. Supplied as a mixture of listed compounds
3. Observed pathway on 2nd PCP addition
4. Hydrogen from yeast extract degradation
5. S.B.=Serum Bottle / B.R.=Batch Reactor
6. Continuous flow stir tank reactor
7. Upflow anaerobic sludge blanket reactor
8. Continuous flow fixed film reactor
9. Granular activated carbon-fluidized bed
10. CH_4 and CO_2 production from ^{14}C-PCP

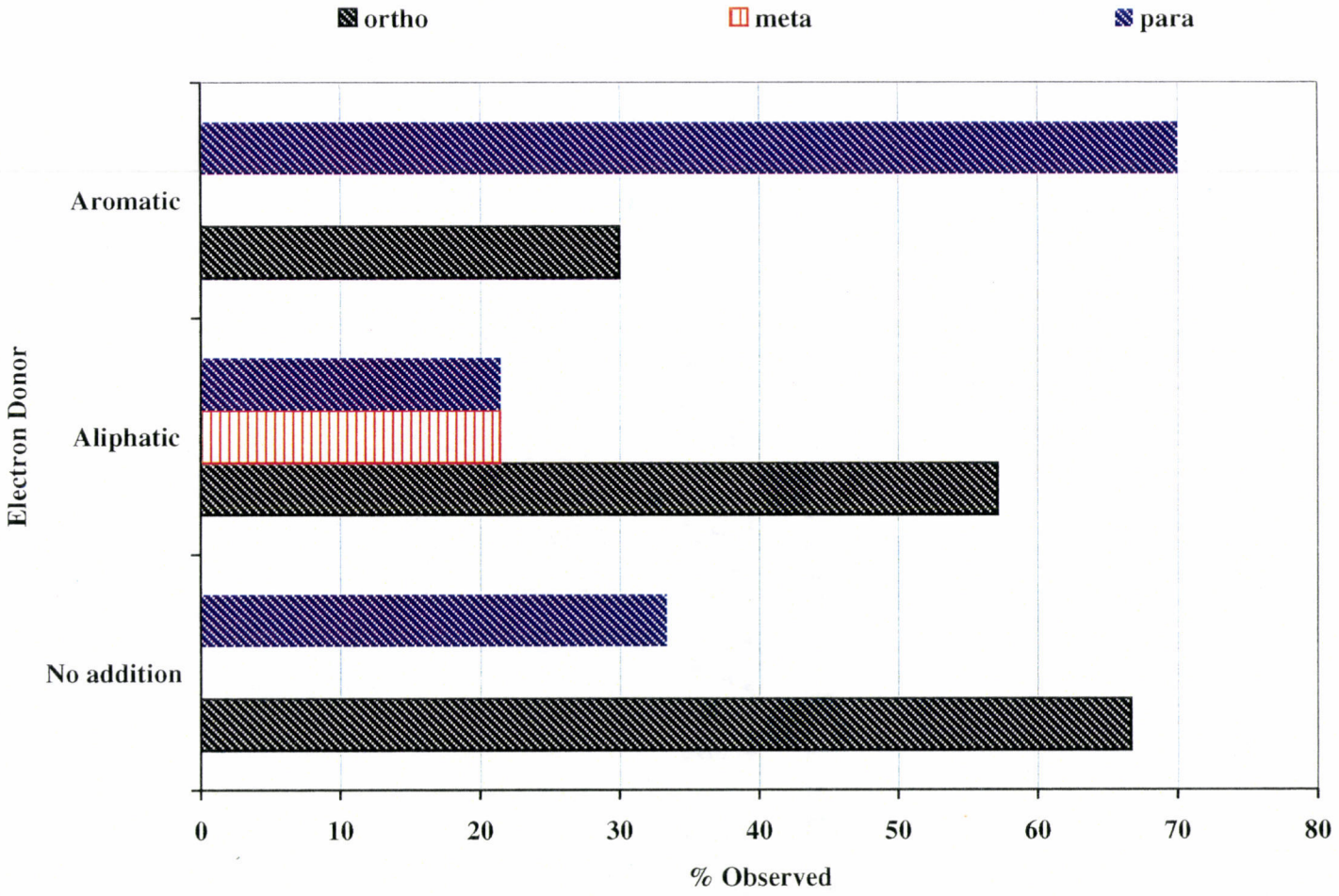


Figure 2.1 Initial position of reductive dechlorination as a function of electron donor structure

conducted by Häggblom et al., 1993a, p-cresol was far more effective than propionate for the support of an anaerobic culture degrading 4-chlorophenol. The differences in culture performance were attributed to the stimulation of population with the ability to degrade the ring structures.

Anaerobic degradation of phenol is known to produce the volatile fatty acids: adipic, caproic, acetic, succinic and propionic (Wu et al., 1993). Syntrophic organisms oxidize the volatile fatty acids to produce hydrogen and carbon dioxide in the presence of acetogens, methanogens or sulfate reducing bacteria. Oxidation of the acids is energetically unfavorable at standard temperature and pressure and will proceed only with the aid of interspecies hydrogen transfer (Brock and Madigan, 1991). Organisms capable of reductive dechlorination compete with methanogens, acetogens, and sulfate reducers for available hydrogen in anaerobic systems. The 3-chlorobenzoate degrading organism *Desulfomonile tiedjei* is believed to use hydrogen and formate as the electron donor in reductive dechlorination (Mohn and Tiedje, 1992). In the study of anaerobic reduction of chloroethenes, (Smatlak et al., 1996) determined that half-velocity coefficients for hydrogen consumption by reductive dechlorinators were nearly 10 times less than that of the methanogens present in the system. The findings support the use of fermentable substrates that yield constant low levels of hydrogen in a mixed anaerobic system. In essence, the low hydrogen levels would allow the dechlorinating population to out compete the methanogens and sulfate reducers for available substrate.

The role of the aromatic electron donor is unclear in the reductive dechlorination of PCP. However, the benefits of aromatic electron donors are many. Fermentation of the aromatic electron donor produces long chain volatile fatty acids, which are slowly degraded yielding a constant hydrogen source (Brock and Madigan, 1991). Several volatile acids were used as electron donors in the observed PCP degradation studies, yet most substrates used did not need the support of syntrophs for oxidation of the acid supplied. Perhaps the use of the smaller volatile acids and easily degradable substrates resulted in higher hydrogen partial pressures that preferentially selected for the growth of sulfate reducers, methanogens or acetogens. Each of these organisms has the ability to out compete dechlorinators at high hydrogen concentrations, resulting in dechlorination rates that are slow or non-existent.

Disparity among electron donor systems and observed degradation pathways has led to an investigation of the behavior of aromatic electron donors for the support of PCP degrading cultures. Column studies conducted by Ellis, 200x with a PCP degrading culture showed that in the presence of a phenol, PCP was degraded initially at both the *ortho* and *para* positions. Dechlorination at the *ortho* position results in 3,4,5-TCP production. However, unlike the acetate systems studied by Cole, 1993 and Stuart, 1996, anaerobic degradation continues through 3,4,5-TCP with phenol. Since different cultures were responsible for PCP transformations, it is unclear what factor initiated the shift in degradation pathways. What remains the most promising finding of the anaerobic phenol-supported system, is the ability to degrade PCP through 3,4,5-TCP. Duff et al., 1995 reported the effectiveness of phenol to support

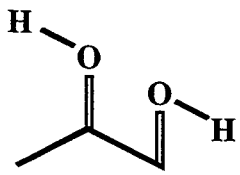
PCP degradation however, 3,4,5-TCP was not an observed intermediate. Hendriksen et al., 1991 observed increased PCP removal rates in fixed film reactors through the addition of glucose to a phenol supported culture. Glucose addition accounted for a higher conversion of PCP to dichlorophenols than control reactors degrading PCP and phenol only. In a similar study, complete dechlorination of PCP occurred in UASB reactors fed phenol and glucose, while PCP transformation in the phenol only control was incomplete (Hendriksen et al., 1992). A mixture of phenol and ethanol was found effective to support the degradation of PCP in serum bottles inoculated with organisms harvested from natural ecosystems and anaerobic digesters (Larsen et al., 1991).

The similarity of PCP and phenol may contribute to its success as an electron donor for PCP degradation. Studies conducted by Godsy et al., 1986 showed that PCP at concentrations greater than one mg/L were inhibitory to the methanogenic fermentation of phenol, while PCP dechlorination seemed unaffected. Phenol is an excellent electron donor for the reductive dechlorination of PCP, however it carries several regulatory responsibilities that make its use questionable in the design of a bioremediation system for PCP contaminated materials. Drawing from the success of phenol supported systems, a qualitative survey of phenolic compounds was conducted to determine their suitability for use in a PCP bioremediation system. The desire to ultimately demonstrate a field-based remediation technology for PCP-contaminated ground water put the emphasis on selection of an electron donor that was acceptable to the regulatory community.

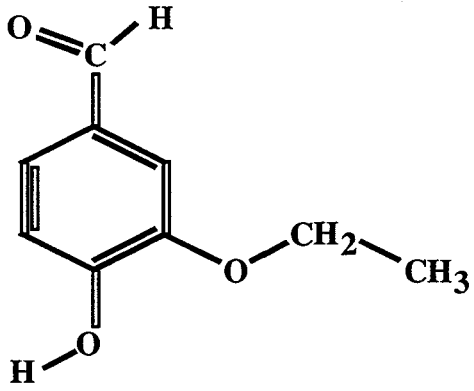
Literature screening of potential substrates began with a search of the Code of Federal Regulations (CFR). Broken down by source and permissible use, 21 CFR Part 182.10 to 182.20 lists several natural spices, seasonings, flavors and essential oils that are "Generally Recognized as Safe" (GRAS) by the Food and Drug Agency (FDA) (CFR, 1995). Chemical compositions of these substances are highly varied and are characterized by complex mixtures of hydrocarbons, alcohols, aldehydes, ketones, esters, acids and phenols. Based on the success of phenol in our laboratory studies, weight was given to spices and flavorings containing the highest percentage of phenolic compounds. Spices or essential oils meeting this criteria included allspice, clove, cinnamon leaves and vanilla. Thirty-six phenolic compounds have been identified in vanilla extract, which was more than double any of the other spices or oils investigated (Richards, 1991). Pure vanilla extract is expensive and infeasible for use in a field scale project. However, the synthetic derivative, imitation vanilla flavoring, shows potential for use in an anaerobic PCP bioremediation strategy.

Imitation vanilla flavoring is composed of a mixture of guaiacol (o-methoxy phenol), ethyl vanillin (4-hydroxy-3-ethoxy-benzaldehyde), sodium benzoate and propylene glycol in water. Chemical structures of the mixture are summarized in Figure 2.2. Three of the four components of imitation vanilla flavoring are aromatic in nature. The fermentation of benzoate has been observed and characterized by many in anaerobic systems (Fang et al., 1997; Li et al., 1996; Liu and Fang, 1997; Zhang and Wiegel, 1990).

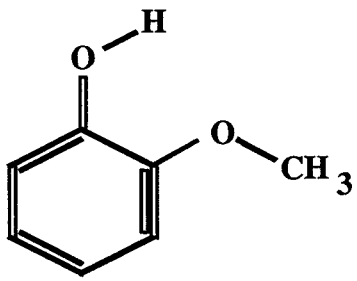
(Woods, 1985) has also described the anaerobic removal of guaiacol. Propylene glycol can support the growth of a wide range of chemorganotrophs and can be degraded through fermentation to acetate (Brock and Madigan, 1991).



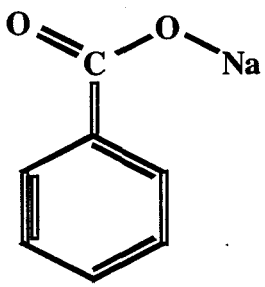
Propylene Glycol
(1,2-Propanediol)



Ethyl Vanillin
(3-Ethoxy-4-Hydroxybenzaldehyde)



Guaiacol
(2-Methoxy Phenol)



Sodium Benzoate
(Benzoic Acid)

Figure 2.2 Structural composition of imitation vanilla flavoring components

Commercially prepared imitation vanilla flavoring is a potent source of carbon and potential energy for microorganisms. The appearance of imitation vanilla flavoring differs among manufacturers. It is commonly found in clear or caramel colored varieties. COD measurements made on the clear variant showed the mixture contained nearly 25,000 mg COD/L. Imitation vanilla flavoring had many desirable properties for use in a field-scale biodegradation study. The flavoring mixture or any of its individual components were GRAS compounds. As such, it was felt that regulatory opposition to the injection of GRAS compounds into a PCP contaminated aquifer would be minimized. From an engineering perspective, the high COD of the flavoring mixture was desirable as it effectively eliminated the need to store large volumes of electron donor on site. What remained unclear was the effectiveness of imitation vanilla flavoring to support an anaerobic culture with a preference for initial *para* dechlorination of PCP.

MATERIALS AND METHODS

Batch serum bottle assays were conducted to evaluate the potential for imitation vanilla flavoring to serve as an electron donor in the reductive dechlorination of PCP. The tests were conducted in duplicate. Parallel controls were used to evaluate the potential for abiotic PCP removal. The serum bottles were inoculated with an anaerobic PCP degrading culture, imitation vanilla flavoring, and a trace inorganic nutrient and vitamin solution modified from (Owen et al., 1979). Progress curves constructed over the experiment duration were used to monitor chlorophenol degradation rates and pathways. Selected components of the imitation vanilla were also monitored to ensure the systems were not electron donor limited. Finally, gas production in the active and control bottles was measured on a volumetric basis.

Inoculum

Consortia used in the serum bottle assay were harvested from a municipal wastewater treatment facility in Corvallis, Oregon. The anaerobic culture originated as a combination of return secondary sludge from an activated sludge system and supernatant from the anaerobic sludge digester. Liquid cell suspensions from each environment were mixed 50/50 on a volume basis. The anaerobic culture was then transferred to an airtight glass container and stored under a nitrogen headspace. The aerobic culture was transferred to a glass vessel where a diffuser stone and compressed laboratory air was used to maintain aerobic conditions. The anaerobic and aerobic systems were covered to prevent phototrophic growth and stored side by side at $21 \pm 2°C$.

The anaerobic system served as a "master" culture source for preliminary electron donor evaluation studies and chlorophenol degradation experiments. The culture was acclimated and maintained with bi-monthly additions of PCP, imitation vanilla, and a modified inorganic nutrient and vitamin solution. The system pH was controlled as needed to maintain approximately neutral conditions. Prior to this study, the consortium was maintained for a period of 10 months.

Experimental System

Amber glass 125 ml serum bottles were used to conduct the degradation study. The bottles were cleaned in a 50% v/v sulfuric acid solution and triple rinsed with de-ionized water. Each bottle contained 35 ml of headspace and 90 ml of liquid. Bottles were capped with Teflon® faced butyl rubber stoppers and 20 mm aluminum crimp seals. Each serum bottle was inoculated with approximately 800 mg VSS, 1 g carbonaceous oxygen demand (COD)/L imitation vanilla flavoring, and 2.25μM PCP. Each bottle was buffered with sodium bicarbonate to ensure neutral to slightly alkaline conditions. Nitrogen and phosphorus were supplied as ammonium chloride and potassium hypophosphate respectively, and trace minerals and vitamins were added in stoichiometric ratios recommended by (Owen et al., 1979).

The pre-sterilized serum bottles were prepared as follows: sodium bicarbonate, vitamins, minerals, and the nitrogen/phosphorus source were added to each serum bottle. The anaerobic inoculum was then quickly added to the system in a liquid slurry form. De-aired distilled water was used for volume makeup and dilution. Cell transfer was conducted in open air. Precautions to minimize oxygen exposure during cell transfer were taken and each vessel that contained cells was continuously purged with nitrogen. The gas allowed the development of a nitrogen blanket in the headspace over the liquid contents of the transfer vessels and serum bottles. The serum bottles were then plugged with Teflon® faced butyl rubber stoppers and sealed with 20 mm aluminum crimp seals. Bottles designated, as experimental controls were heat sterilized on two consecutive days.

Capped serum bottles were purged with nitrogen, and sampled for chlorophenols prior to donor addition. Pressure in each bottle headspace was equalized to atmospheric following the purge with a new disposable 22-gauge syringe needle. Disposable needles were chosen to reduce the chance of cross contamination or accidental inoculation of the experimental controls. A solution of PCP in imitation vanilla flavoring was then added to the system to start the experiment. Bottles had an initial liquid concentration of imitation vanilla flavoring at 1g COD/L and 2.25μM PCP. Following donor addition, the bottles were shaken and sampled for chlorophenols. To alleviate positive pressure generated in donor addition, the headspace in each bottle was equalized to atmospheric pressure. Bottles were then inverted and placed on rotary shaker table at 21± 2 °C.

Sampling Procedure

The active bottles and experimental controls were sampled immediately after the addition of the donor and PCP and at 12 hour intervals thereafter. Bottles were removed from the shaker table and allowed to settle for a period of ten minutes prior to sampling. Gas production was measured first with a 5 ml luer tip syringe (Popper & Sons, New Hyde Park, NY). The syringe walls were first lubricated with de-ionized water to allow easy plunger movement. Air present in the syringe barrel was expelled and a new syringe needle was attached. The serum bottle septa were then punctured with the syringe. Displacement of the plunger indicated gas production since the last sampling interval. Liquid samples were collected from the

bottles with 100 µl syringes (Hamilton Co., Reno, NV) and were immediately prepared for chlorophenol analysis.

Chemical Sources

Pentachlorophenol (purity > 99.9%) was obtained from Sigma Chemical Co. (St. Louis, MO) and was used without further purification. Individual components of the imitation vanilla flavoring were obtained from Aldrich Chemical Co. (Milwaukee, WI). All were reagent grade and possessed purity greater than 99%. Other chemicals consumed over the course of the experiment were obtained from Mallinckrodt Co., (Paris, KY) or EM Science, (Cherry Hill, NJ). Chlorophenol analytical standards were obtained from Ultra Scientific Inc., (North Kingston, RI).

Analytical Procedures

Chlorophenol samples were acetylated and extracted into hexane using a modification of the method developed by (Voss et al., 1980) and the National Council of the Paper Industry for Air and Stream Improvement (1981). Extractions were conducted as follows: 500 µl of a solution containing 30.4 g/L K_2CO_3 and 250 µg/L 2,4,6 tribromophenol (an internal standard) was combined with a 100 µl sample from the serum bottles in a disposable glass culture tube with a Teflon® faced cap. 100 µl of acetic anhydride was added and the tube was mechanically shaken for 20 minutes. 1 ml of chromatographic grade hexane was added and the tube was shaken for an additional 20 minutes. Hexane was removed from the tube and transferred to a 2 ml amber glass vial. The vial was sealed with a Viton® faced crimp cap. Vials were immediately loaded for analysis by capillary gas chromatography.

Chlorophenols were quantified on a Hewlett Packard 5890A gas chromatograph. Automated 1 µl injections were made on the inlet, which was operated, in a splitless configuration. A Hewlett Packard 3392A integrator handled acquisition and signal processing from the 63Ni Electron Capture Detector (ECD). Separation of chlorophenol congeners was accomplished on a DB-5 fused silica capillary column (30m x 320µm I.D. x 0.25µm film; J & W Scientific, Folsom, CA). Helium provided at 35 cm/s served as the column carrier gas. A 95/5 blend of argon/methane at 75 ml/min was used for detector make-up. The instrument was operated as follows: initial temperature of 45°C was held for 2 minutes; the temperature was then increased 25°C/min to 140°C and held for 5 minutes; the oven was then increased 5°C/min to 245°C where it was held for 10 minutes.

Solids concentrations in the batch cell cultures were analyzed for total and suspended solids using standard methods 2540D and 2540E (Association, 1989).

RESULTS

The effectiveness of imitation vanilla flavoring as an electron donor was evaluated by measuring chlorophenol concentrations in the experimental controls and biologically active bottles with time. To facilitate presentation of experimental findings, average chlorophenol concentrations from the active or sterile bottle sets are presented.

Pentachlorophenol Transformation

PCP was rapidly removed from bottles containing active biomass and imitation vanilla flavoring. Figure 2.3 represents PCP micromolar (μM) concentration and the cumulative volume of gas produced in the active and sterile serum bottles as a function of time in hours. During the first 12 hours, PCP concentrations within the active system fell from an initial 2.20 to 0.89 μM, representing, a mass removal of approximately 60%. With increasing time, PCP concentrations in the active system continued to decrease and asymptotically approached zero. PCP was removed in the active serum bottles from an initial concentration of 2.20 to 0.029 μM. The observed decrease in serum bottle PCP concentrations represented transformation efficiencies that approached 99%. Initial PCP mass removal approached 40% in the sterile system as the initial concentration fell from 3.59 to 2.19 μM over the first 12 hours. Measurements with time yielded no appreciable reductions in the sterile system PCP concentrations. Samples collected after 12 hours showed PCP concentrations averaged 2.32 $\pm$ 0.12 μM.

Observed Gas Production

Over the 168 hours of sampling, the active system produced 10.6 ml of gas. Observed gas production was nearly three times greater than the theoretical yield if the concentration of donor supplied was transformed completely to methane. Greater than 40% of the total gas produced evolved during the first 12 hours. With increasing time, gas production volumes decreased. Gas composition of the active system was not evaluated. There was no measurable gas production in the sterile system.

Observed Transformation Pathway

Chlorophenol concentrations as a function of time in the active system are shown in Figure 2.4. Immediate removal of PCP, resulted in the production of PCP's *ortho* dechlorination product 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP). Production of the *para* dechlorination species 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) was also observed but at reduced concentrations. Corresponding to PCP removal, an increase in 2,3,4,5-TeCP concentrations increased from 0 to 0.60 μM and 2,3,5,6-TeCP from 0 to 0.11 μM. Maximum concentrations of 2,3,5,6-TeCP and 2,3,4,5-TeCP were observed at hours 20 and 28 of the study. Complete removal of 2,3,5,6-TeCP and 2,3,4,5-TeCP occurred by hours 84 and 168, respectively. Dechlorination products of 2,3,4,5-TeCP and 2,3,5,6-TeCP were observed early in the experiment at hour

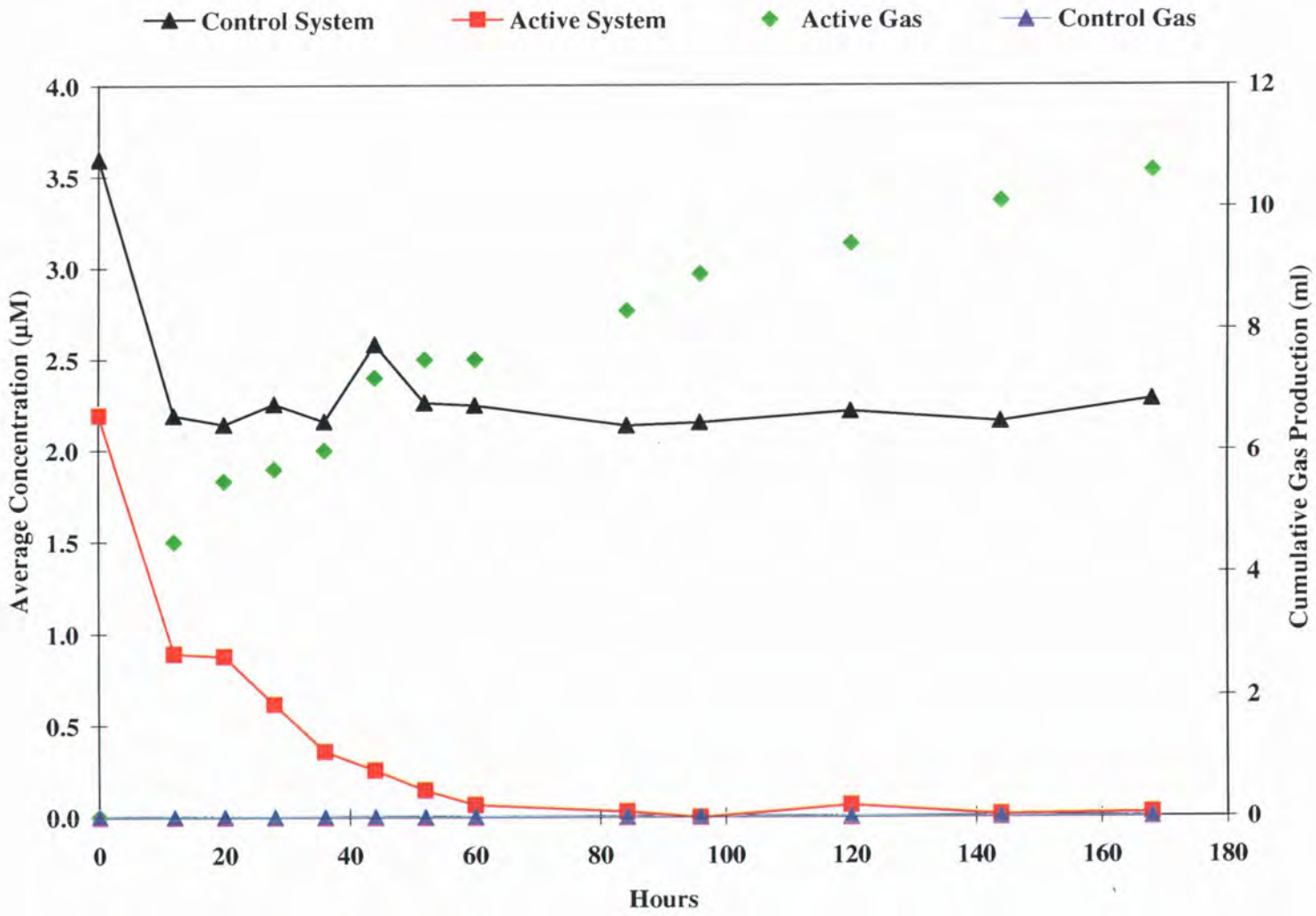


Figure 2.3 Pentachlorophenol concentration as a function of time

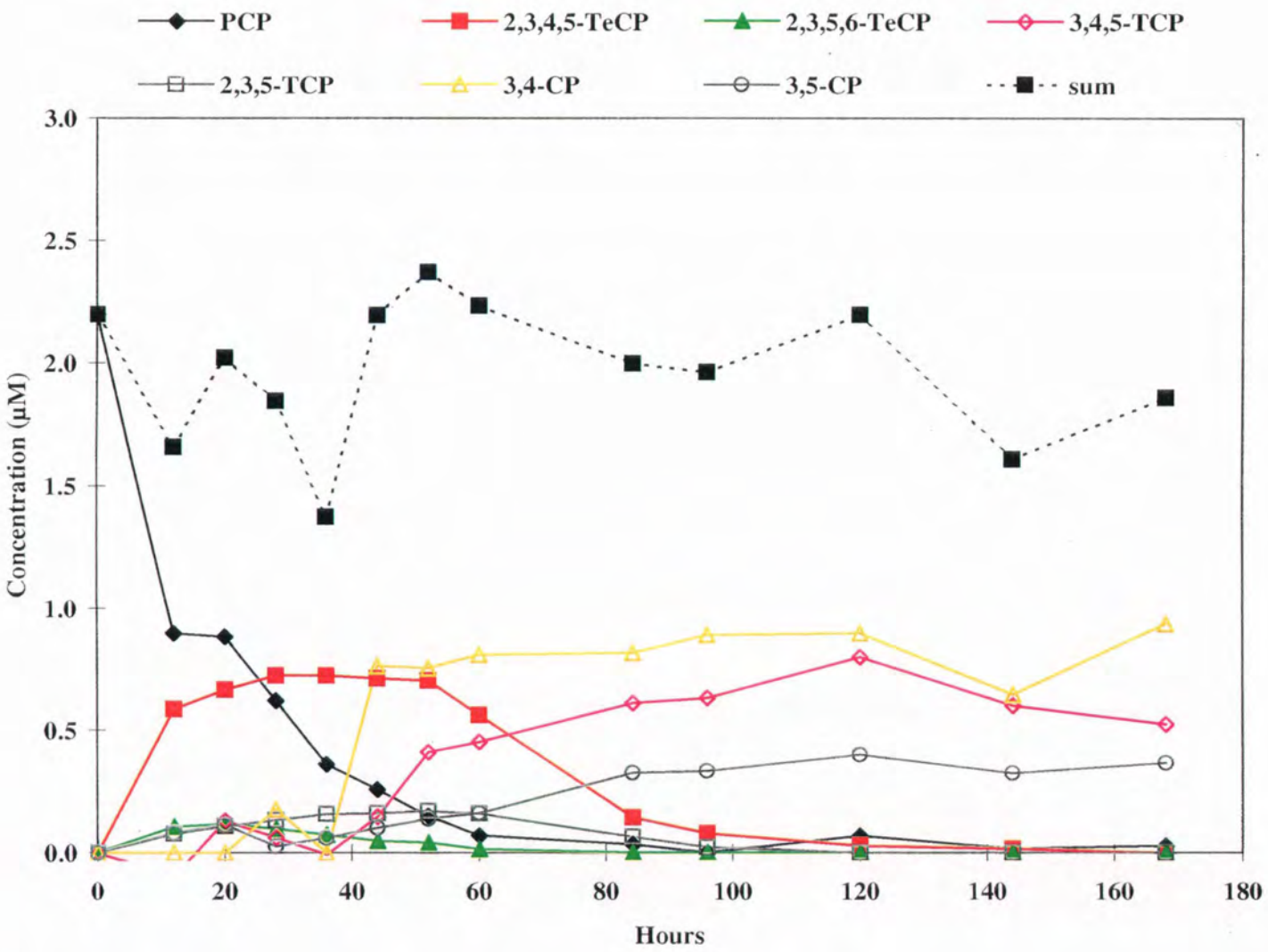


Figure 2.4 Average active system chlorophenol concentration as a function of time

12. Transient production of 2,3,5,6-TeCP's *ortho* dechlorination product 2,3,5-trichlorophenol (2,3,5-TCP) was observed from 12 to 120 hours. Degradation of 2,3,4,5-TeCP at the *ortho* position forming 3,4,5-trichlorophenol (3,4,5-TCP) commenced at hour 20. Concentrations of 3,4,5-TCP steadily increased before reaching a plateau at 120 hours. With increasing time, 3,4,5-TCP concentrations decreased slightly however, complete removal was not observed over 168 hours of sampling. Production and accumulation of 3,4-dichlorophenol (3,4-DCP) and 3,5-dichlorophenol (3,5-DCP) began at hour 28. No further degradation products were observed in the active system.

Figure 2.5 summarizes the observed metabolites of PCP degradation when imitation vanilla flavoring serves as an electron donor. Where appropriate, solid lines depict observed transformation products. Since the pathway shown in Figure 2.5 was not developed with individual compound degradation tests, alternate pathways are shown by dotted lines. PCP was removed from the active system by initial dechlorinations at the *ortho* and *para* positions. The *ortho* product, 2,3,4,5-TeCP was dominant. However, measurable quantities of the *para* product, 2,3,5,6-TeCP were detected. Dechlorination of 2,3,4,5-TeCP at the *ortho* position resulted in the production of 3,4,5-TCP. Removal of a *meta* chlorine from 3,4,5-TCP produced 3,4-DCP. Production of 2,3,5-TCP is speculated from an *ortho* dechlorination of 2,3,5,6-TeCP however, a *para* dechlorination of 2,3,4,5-TeCP also may produce 2,3,5-TCP. The parent of 3,5-DCP is unknown, as it may have originated from either 3,4,5-TCP or 2,3,5-TCP or both.

Evidence of Pentachlorophenol Biotransformation

Chlorophenol concentrations present in the sterile system as a function of time are shown in Figure 2.6. Over the first sampling interval, a decrease in PCP concentrations was observed in the sterile bottles. Concentrations remained constant and averaged 2.23 ± 0.12μM after the initial decrease observed at 12 hours. Residual chlorophenols present in the inoculum 3,4,5-TeCP, 2,3,5-TCP, 3,4-DCP and 3,5-DCP were not removed from the system with time. There was no evidence to support biological transformation of any chlorophenol present in the sterile system.

Figure 2.3 elucidates the removal of PCP from the active system supplemented with imitation vanilla flavoring whereas; sterile system concentrations remained nearly constant over the duration of the experiment. In both sterile and active systems, PCP behavior is nearly identical over the first 12 hours of the experiment. After 12 hours, no appreciable change in PCP concentrations was observed in the sterile system. However, PCP concentrations in the active system continued to decrease with increasing time. The absence of gas production in the sterile system also lends support to biological PCP transformation when compared to the evolution of nearly 11 ml of gas in the active system.

Perhaps more important than the removal of PCP shown in Figure 2.3, is the production of dechlorinated metabolites (Figure 2.4) and the absence of metabolite production in the sterile system (Figure 2.6). The figures clearly show that reductive biological processes are responsible for PCP

degradation in the active bottles. Removal of PCP, correlates well the observed production and ultimate removal of two tetrachlorophenols (Figure 2.4), which were not initially present in the active system. Similar trends were observed in the removal of several of PCP's dechlorinated metabolites. In the active system (Figure 2.4), a mass balance around PCP and observed dechlorination products showed an average chlorophenol concentration of 1.96 ± 0.29 μM. Comparing mass balance measurements to 2.20 μM, the initial PCP molar mass, 89 % of the chlorophenol mass can be accounted for over the experiment duration. Chlorophenols present in the sterile system (Figure 2.6) remain virtually unchanged over the duration of the experiment.

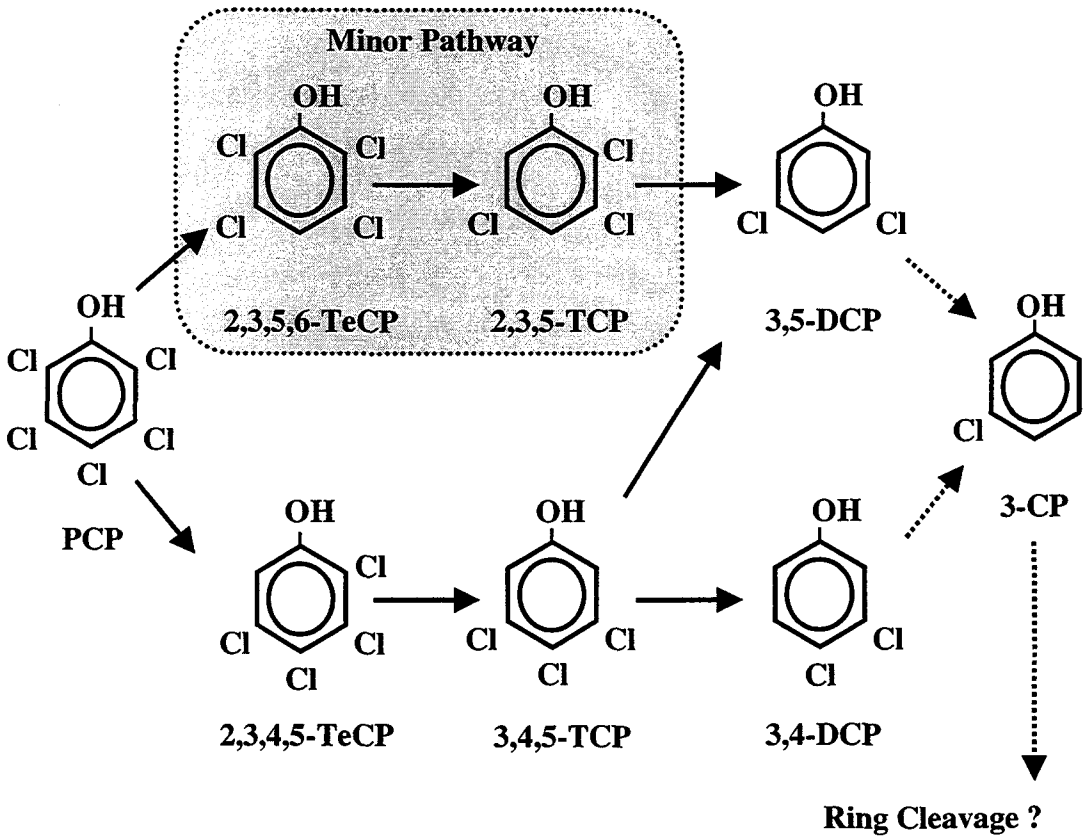


Figure 2.5 Observed pentachlorophenol degradation pathway

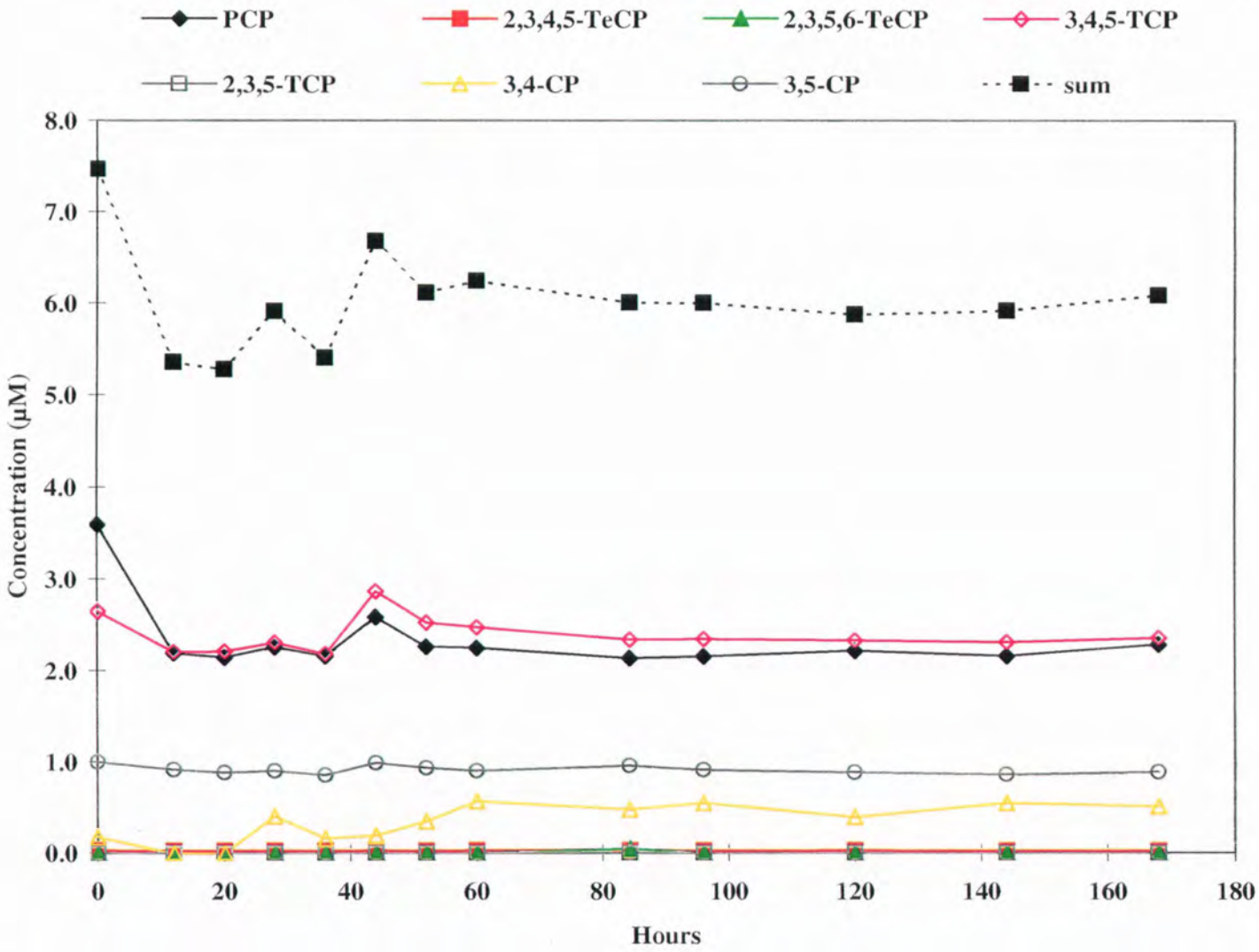


Figure 2.6 Average sterile system chlorophenol concentrations as a function of time

Observed Transformation Rates

Rates of reductive dechlorination were calculated from metabolite production and removal in the active bottle system. Employing batch kinetic analysis, PCP concentrations as a function of time were evaluated for reaction order. First order degradation mechanisms correlated well with measured PCP removal in the active system. Performance of the active system was modeled as a combination of first order parallel and series reactions. First order rate equations were developed to account for microbial transformation of the parent compound and production of a single metabolite in a batch system. Loses due to mechanisms other than biological transformations were assumed negligible. Solutions to rate expressions describing first order reactions in parallel and series were determined graphically from experimental data using methods outlined by Levenspiel, 1972.

Since PCP degradation was responsible for the production of 2,3,4,5-TeCP and 2,3,5,6-TeCP, the reactions were assumed to proceed by two parallel pathways. Using experimental data collected during the production phases of 2,3,4,5-TeCP and 2,3,5,6-TeCP and the overall rate of PCP degradation, rates of metabolite production were computed. The rate of metabolite production was used to estimate the rate of removal as a function of the maximum observed concentration. Assuming reductive dechlorination processes governed the rate of observed tetrachlorophenol removal may also describe the rate of trichlorophenol production. Estimates of degradation rates were only made for PCP transformation through trichlorophenols observed. Experimental rate observations are summarized in Table 2.2.

Table 2.2 Observed First Order Degradation Rates

Observed Transformation	**Dechlorination Position**	**First Order Rates (hour^{-})**	
		Production	**Removal**
PCP $\rightarrow$ 2,3,4,5-TeCP + 2,3,5,6-TeCP	*Ortho, Para*		0.0514
PCP $\rightarrow$ 2,3,4,5-TeCP	*Ortho*	0.0442	0.0549
PCP $\rightarrow$ 2,3,5,6-TeCP	*Para*	0.0072	0.1163
2,3,4,5-TeCP $\rightarrow$ 3,4,5-TCP	*Ortho*	0.0549	≈ 0
2,3,5,6-TeCP $\rightarrow$ 2,3,5-TCP	*Ortho*	0.1163	≈ 0

DISCUSSION

Pentachlorophenol Degradation

Like many laboratory studies, anaerobic PCP degradation studies were conducted as a precursor to pilot and field scale projects to obtain a better understanding of microbial transformation processes. Batch bottle assays proved an acceptable method for the evaluation of substrate suitability. Imitation vanilla flavoring appears to be an effective electron donor for the support of a PCP degrading anaerobic consortium. In an effort to minimize inhibition due to PCP or any of its metabolic products, initial PCP loading in the active bottle system was purposely kept low. The treatment studies were conducted at an initial PCP concentration of 2.20 μM. The concentration was roughly half of average PCP values found in ground water at a nearby wood preserving facility for which, field studies were planned. Based on the rapid rate of removal exhibited in the active systems (Figure 2.3), removal of higher PCP concentrations is possible.

Unfortunately, the relationship between the observed rate of PCP degradation and the chemical structures of the donor mixture or donor concentration was not determined. The system studied contained high levels of COD harnessed within a complex mixture of multiple substrates. It is likely that degradation of propylene glycol, sodium benzoate, guaiacol and ethyl vanillin likely proceeded by fermentative pathways. Within the mixed microbial community, fermentation generally yields hydrogen and carbon dioxide, which in turn are consumed by methanogenic and acidogenic organisms to produce methane and acetate. The relationship among dechlorinators and the anaerobic consortium is complex. Many have proposed that hydrogen is the true electron donor used in the process of reductive dechlorination (Maymo-Gatell, 1995; Mohn and Kennedy, 1992; Smatlak et al., 1996; Zhang and Wiegel, 1990). The success of reported dechlorination when hydrogen serves as the electron donor seem to correlate with substrates that are slowly degraded and only under low partial pressures of hydrogen. Slow release of hydrogen may have occurred by the degradation of the aromatic and aliphatic mixture of electron donors present in the imitation vanilla flavoring.

Of the compounds in imitation vanilla flavoring, propylene glycol is present in the highest concentration. Comparatively it also has the most basic chemical structure. Based upon the structure and concentration, it is assumed in a degradation hierarchy that the propylene glycol would be rapidly exhausted. Slow degradation of the structurally complex aromatics remaining would then follow. This concept may explain the rapid evolution of headspace gas during the first 12 hours of the study and the relatively slower gas production thereafter. Assuming the COD supplied by the donor was converted solely to methane (3.5 ml), the total production of gas in the active system (10.6 ml) was much higher than expected. Gas composition was not evaluated in the experiment but it is possible that methanogenesis was inhibited or was very slow. The absence of methanogenesis would allow the buildup of carbon dioxide and hydrogen generated in substrate degradation which, may account for the greater than expected gas volumes.

Reductive Dechlorination Pathways

While many types of anaerobic cultures have been shown to degrade PCP when supplied an electron donor, the degradation pathways are often very different. Evaluation of the individual components of the imitation vanilla flavoring was not undertaken therefore, it is unclear what roles the compounds played in the overall degradation of PCP. Dechlorination of PCP in the active system primarily proceeded through an initial *ortho* dechlorination of PCP. A much smaller percentage was observed as the *para* dechlorination product. The split pathway was shown by several groups. However, initial dechlorination at the *para* position was dominant (Beaudet et al., 1998; Hendriksen et al., 1992; Juteau et al., 1995a; Larsen et al., 1991). Regardless of the initial dechlorination position, the imitation vanilla supported culture showed the ability to dechlorinate at all positions as was evidenced by the presence of 2,3,4,5-TeCP, 3,4-DCP and 2,3,5,6-TeCP. It remains unclear whether a dechlorination at 3,4,5-TCPs *para* position or 2,3,5-TCPs *ortho* position was responsible for the observed production of 3,5-DCP. In a variety of experimental systems, literature supports the production of 3,5-DCP from both 3,4,5-TCP and 2,3,5-TCP (Juteau et al., 1995a; Larsen et al., 1991; Liu et al., 1996; Madsen and Aamand, 1991; Mikesell and Boyd, 1985).

Experimental Controls

In both experimental systems, PCP exhibited nearly identical behavior during the initial 12 hours (Figure 2.4 and Figure 2.6). The reason for the rapid initial decrease in the sterile system is unclear. However, it is possible that changes induced by sterilization methods rather than biological mechanisms were responsible for the observed PCP removal. This conclusion can be supported by several concepts. During 168 hours of sampling in the sterile system (Figure 2.6), no production of dechlorinated intermediates was observed. In the sterile system, periods after 12 hours show PCP concentrations average $2.23 \pm 0.12\mu M$. The average of the sterile system corresponds well with initial PCP concentrations (2.20 μM) measured in the active system. The sterile and active systems were constructed in exactly the same manner and each contained about the same initial concentration of biomass and PCP. Initial decreases in PCP and 3,4,5-TCP could also be explained by sorption to the dead biomass. When compared to the behavior of 3,5-DCP, this idea is plausible as both PCP and 3,4,5-TCP has greater lipophilic tendencies and consequently lower aqueous water solubility. Furthermore, aqueous chlorophenol samples were not filtered before extraction and it is possible that cell mass lysed, in sterilization, contained high concentrations of PCP which may have skewed the initial data point.

Observed Transformation Rates

All but one of the reductive dechlorinations subjected to rate analysis occurred in the *ortho* position. Comparison of relative dechlorination rates between the *ortho*, *meta*, and *para* positions was not possible due to the degradation pathways observed. Substitution of the calculated rate constants to first order degradation expressions as a function of time allowed the generation of theoretical degradation curves (Figure 2.7 and Figure 2.8). Figure 2.7 depicts theoretical degradation of PCP and the associated

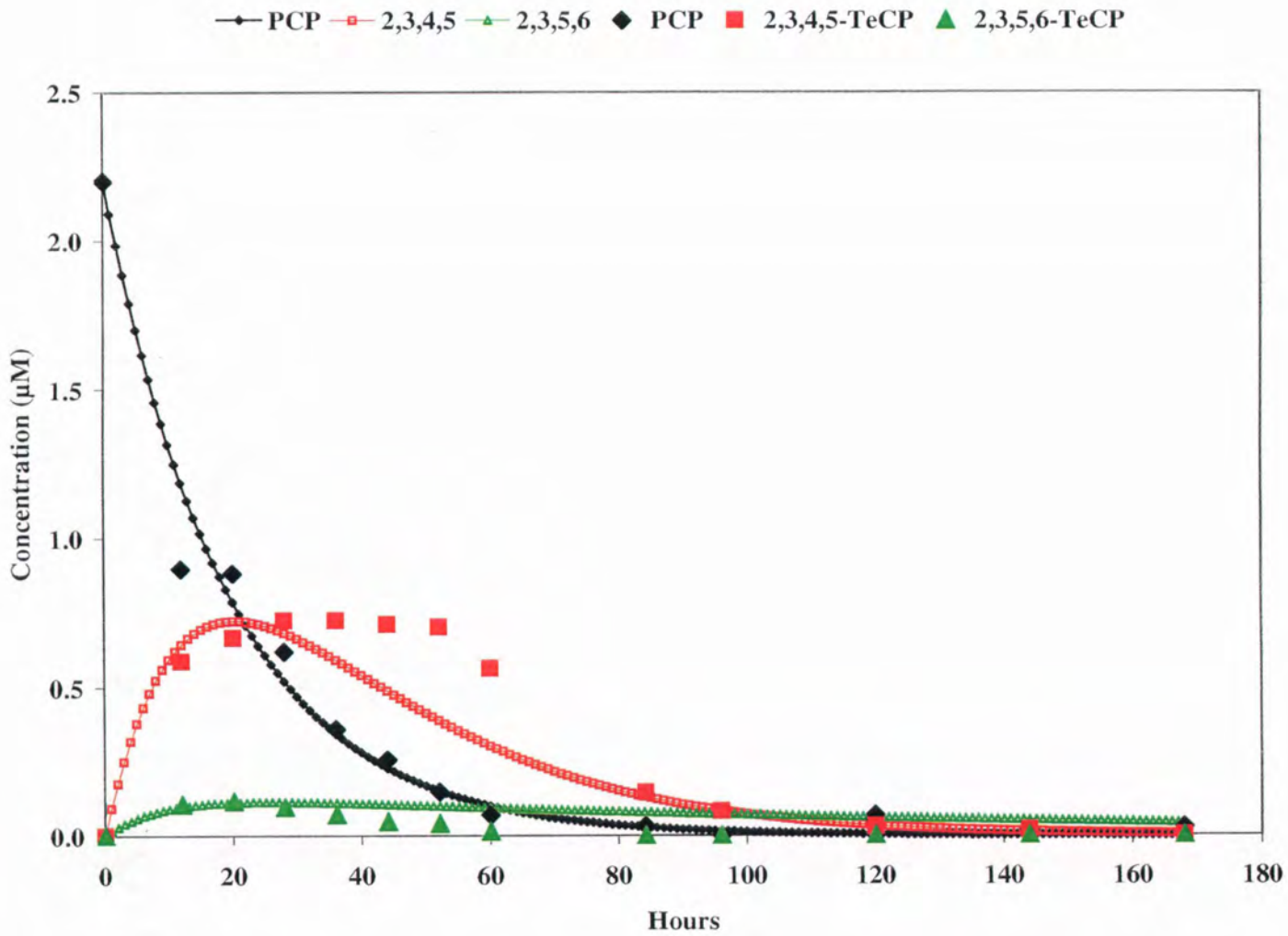


Figure 2.7 Pentachlorophenol to tetrachlorophenol model prediction

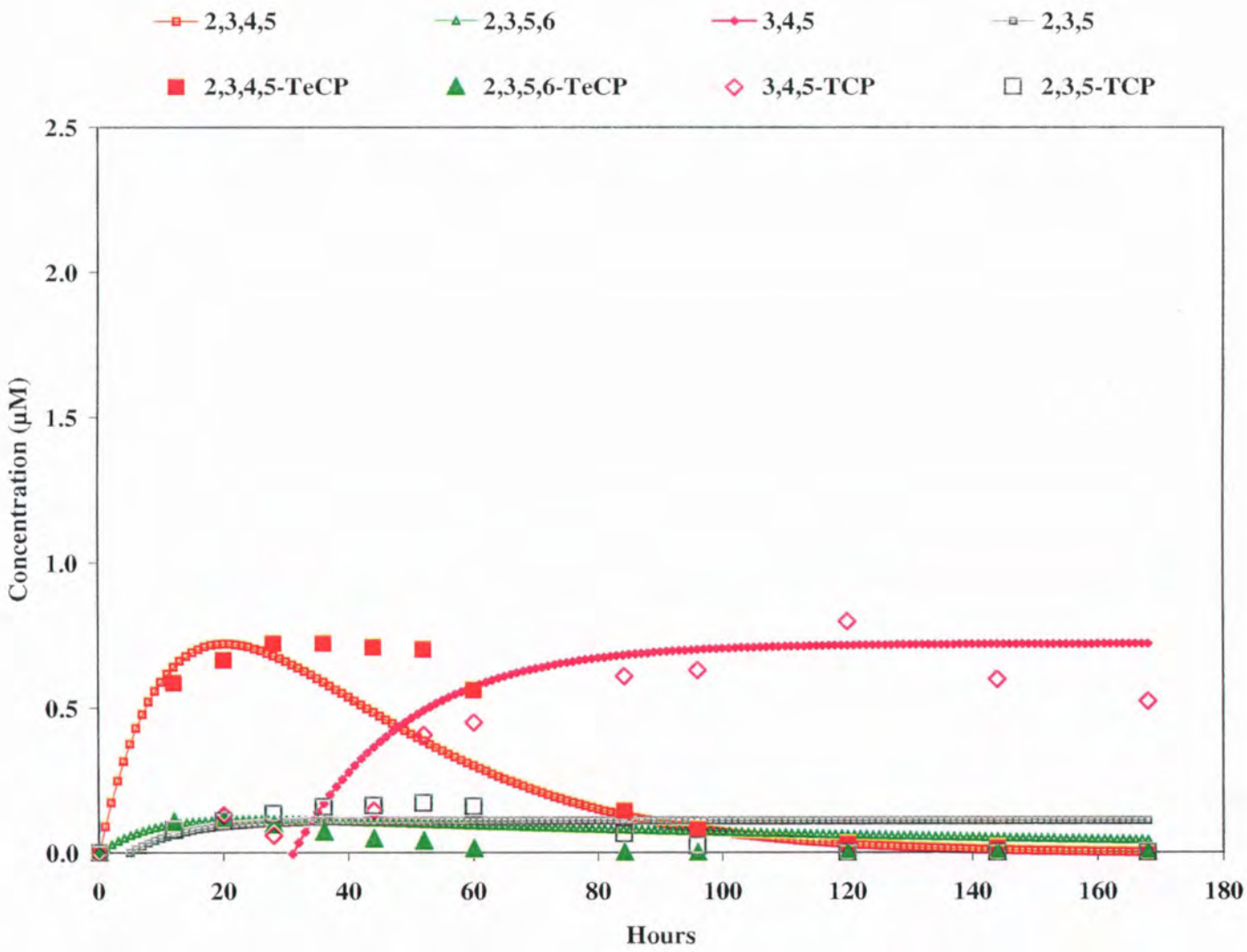


Figure 2.8 Tetrachlorophenol to trichlorophenol model prediction

production of the two observed metabolites, 2,3,4,5-TeCP and 2,3,5,6-TeCP. Solid lines represent theoretical data while; solid symbols represent experimental data. The calculated degradation coefficients describe PCP removal very well. Although the model of PCP metabolite production follows the general trend observed in the experiment, it fails accurately predict the all of the experimental data. The model employed over predicts the maximum concentrations of both tetrachlorophenols at premature times. Concentration profiles of 2,3,4,5-TeCP and 2,3,5,6-TeCP exhibit similar trends. Production of the metabolites is immediate at concentrations approaching the maximum observed. This behavior results in a pronounced plateau, which is apparent in the progress curves of 2,3,4,5-TeCP and 2,3,5,6-TeCP (Figure 2.4). It is plausible that some type of inhibition of 2,3,4,5-TeCP and 2,3,5,6-TeCP exists based on the observed experimental data and the lack of correlation by the mathematical model. From 28 to 52 hours, 2,3,4,5-TeCP concentrations are relatively constant despite continued decreasing PCP concentrations. Between 52 and 60 hours, rapid degradation of 2,3,4,5-TeCP occurred at corresponding PCP concentrations from 0.15 to 0.07 μM. Degradation of 2,3,5,6-TeCP was also temporarily halted at a maximum concentration from 12 to 28 hours. Formation of an accumulated product plateau by the model however was not as evident. Further investigation of PCP degradation mechanisms is needed to fully support a model incorporating attributes of competitive inhibition.

Model results when 2,3,4,5-TeCP and 2,3,5,6-TeCP serve as the parent compounds are shown in Figure 2.8. Solid lines represent mathematically derived concentrations whereas; solid symbols represent experimental data. Despite the fair correlation of 2,3,4,5-TeCP behavior with that of the model, concentration profiles and mathematical predictions of its *ortho* product 3,4,5-TCP agree nicely. Predictions of 2,3,5,6-TCP product, 2,3,5-TCP are shifted slightly in time frame. The shift is likely an artifact of the poor fit generated in the PCP to 2,3,5,6-TeCP model. The inability of the mathematically derived predictions to accurately describe the removal of 2,3,5,6-TeCP and 2,3,5-TCP may be attributed to the nature of the model, scientific assumptions and simplifications applied to the system. Microbial growth was neglected in the model presented. Therefore, it is not surprising that the observed experimental removal of intermediate products of PCP reductive dechlorination were faster than mathematically predicted values.

SUMMARY AND CONCLUSIONS

In the development of a biological treatment process for PCP contaminated groundwater, the effectiveness of novel electron donor, imitation vanilla flavoring was evaluated. The following observations were made in serum bottle assays. (1) Imitation vanilla flavoring is an effective electron donor for anaerobic PCP reductive dechlorination. (2) When supplied as the electron donor, imitation vanilla flavoring was consumed in the biological transformation of PCP. (3) PCP reductive dechlorination was catalyzed at all chlorine substituted positions; limited accumulation of 3,4,5-TCP was observed in the

experimental system. (4) Transformation of PCP was rapid; 99% of the initial PCP mass was transformed in less than 85 hours. Overall, the results of this study indicate that imitation vanilla flavoring would be an effective electron donor for the biological treatment system currently under development.

The utility of biological processes for the remediation of contaminated groundwater has been realized. However, the additional regulatory burden associated with the injection of chemicals requisite for *in-situ* biological treatment detracted from the attractiveness of full-scale treatment systems. The potential application of imitation vanilla flavoring to groundwater remediation is promising. Although many electron donors are capable of supporting PCP degrading cultures, the physical and chemical properties of imitation vanilla flavoring are ideal for field scale applications. Furthermore, the individual components of the electron donor mixture are GRAS by the FDA. It is envisioned that injection of chemicals GRAS to an aquifer system may have wider acceptance among the regulatory community and adjacent property owners. There is a wide array of compounds GRAS. Therefore, their application as electron donors shows promise for the continued development and implementation of bioremediation systems.

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CHAPTER 3

PILOT SCALE DEMONSTRATION OF A PERMEABLE BARRIER TECHNOLOGY FOR THE *IN-SITU* BIOREMEDIATION OF PENTACHLOROPHENOL-CONTAMINATED GROUND WATER

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ABSTRACT

A pilot scale demonstration of a biological permeable barrier was conducted in a pentachlorophenol-contaminated aquifer at a wood preserving facility. A permeable reactor was constructed to fit within a large diameter well. Arranged in series, a cylindrical reactor 24'' x 36'' (0.61 x 0.91m) (diameter x height) was partitioned to provide one anaerobic and two aerobic treatment zones. Mixing zones precede each biologically active zone to provide the opportunity for nutrient injection and gas lift mixing. A mixed microbial consortia supported on ceramic saddles was used to inoculate both the anaerobic and aerobic treatment zones. Environmental conditions were monitored with two continuous flow cells capable of pH and oxidation/reduction potential measurements. Aqueous samples were collected from twenty-eight sampling points within the reactor and allowed for the spatial and temporal characterization of biological removal processes.

Biodegradation of pentachlorophenol (PCP) was evaluated under several environmental conditions. There was no evidence to support natural PCP biodegradation. In the presence of the inoculated permeable barrier but without exogenous substrate, PCP present in the groundwater was not degraded. Under oxidizing conditions in the presence of cells and imitation vanilla flavoring, PCP was not appreciable degraded in the permeable barrier reactor. PCP degradation was observed under a reduced environment in the presence of imitation vanilla flavoring and cells. Environmental conditions measured in the treatment zones indicated that PCP biotransformation occurred under anaerobic conditions. Chemical speciation of PCP degradation products indicated reductive dechlorination was the primary mechanism of removal. PCP degradation proceeded by sequential *ortho* dechlorination forming 3,4,5-trichlorophenol. Degradation of 3,4,5-TCP resulted in the production of 3,4-dichlorophenol and 3,5-dichlorophenol, which in turn were completely removed. There was no accumulation of any dechlorinated products in the system. Degradation of PCP *in-situ* was complete in the pilot scale demonstration at the L.D. McFarland facility.

RESEARCH OBJECTIVES

Traditional permeable reactive barriers employing degradation processes catalyzed by zero valent metals are ineffective for the remediation of materials impacted by chlorinated aromatic compounds. The focus of this research was to design and demonstrate a permeable barrier for the biological degradation of highly chlorinated aromatic compounds. The use of biological treatment process rather than abiotic reduction is a significant deviation in current permeable barrier research and field applications. Through this deviation, it was hoped to expand the realm of contaminates applicable to permeable barrier treatment strategies. Having previously identified a conceptual biological treatment regime for pentachlorophenol (PCP) impacted groundwater, the design of a biological permeable barrier was undertaken. For many reasons, PCP was a desirable demonstration compound. It is chemically stable, persistent in soil and

groundwater, widely distributed in the environment and biological degradation mechanisms are similar to other halogenated synthetic organic compounds. In an effort to develop a passive *in-situ* biological treatment strategy for PCP-contaminated groundwater, this study was undertaken setting forth the following goals:

- To develop a passive bioremediation system for ground water interception and design a functional, cost effective experimental system to conduct *in-situ* PCP biodegradation studies.
- To design a removable permeable barrier reactor with the capacity for process sample collection, mixing, nutrient injection, and ample surface area for cellular growth.
- To characterize the *in-situ* removal of PCP, a model compound in a biological permeable barrier reactor.

INTRODUCTION

In-situ bioremediation schemes often fail because a suitable substrate, the contaminant and viable microorganisms lack adequate mixing in the subsurface. Successful bioremediation projects often rely on pump and treat configurations with process reactors above ground. Above ground reactors offer good process control but suffer from high operation and maintenance costs. Efforts to increase the success of field based bioremediation systems have resulted in an array of technologies for subsurface treatment. Unfortunately, many of these technologies center on extraction and injection or infiltration methods to stimulate biological removal of the contaminant. In several cases, these technologies have been shown very effective for *in-situ* groundwater remediation (Dybas et al., 1998; Gersberg et al., 1995; Hooker et al., 1998; Hopkins and McCarty, 1995; Hopkins et al., 1993). Traditional treatment systems, however, possess inherent flaws: energy input for pumping the ground water, long term operation and maintenance costs, and the regulatory issues involved with reinjection limit their desirability for field scale bioremediation.

One of the biggest challenges in the use of field-scale bioremediation strategies is adequate mixing of contaminant, organisms and substrate. The use of pump and injection systems to recirculate groundwater offers the potential to amend water returning to the aquifer with nutrients to support biological growth. Mass transfer limitations, retardation and reaction of the nutrients within the aquifer structure, limit the effectiveness of stimulating the growth of organisms equally distributed in the contaminant plume. Within the areas of injection, degradation is often adequate. Yet, in areas outside of the stimulated zones, contaminants migrating with the subsurface plume evade capture and treatment. Over time, growth of extraneous organisms at the injection source will eventually rob the mixing efficiency and the effective radius of biological influence. In many systems, the problem of biological well fouling is severe. Pulsed nutrient addition or time release compounds may help to alleviate fouling at the injection source and increase the efficiency of treatment in pump and injection systems (Chapman et al., 1997; Peyton, 1996).

Field based treatment systems employ a variety of methods to supply nutrients to the biological population. Nutrients may be supplied to shallow unconfined aquifers by recharge with nutrient rich water applied to the ground surface. Field scale application of this technology by Hutchins et al., 1998; and Sweed et al., 1996 was demonstrated for benzene, toluene, ethyl benzene and xylene (BTEX) removal under denitrifying conditions. Surface nutrient addition was made with fresh water; dilution of the dissolved BTEX components in the aquifer was noted. The effectiveness of the technology is limited by the depth to the water table, competition for applied nutrients in the unsaturated zone and the supply of large volumes of clean water. Employing the concept of surface irrigation, O'Leary et al., 1995 used drip irrigation equipment to supply a synthetic BTEX contaminated groundwater to a large soil plot. When supplemented with nitrate, BTEX was completely removed while percolating through the unsaturated soil. The study was an effective demonstration of an in-ground biological trickling filter cable of BTEX degradation. Surface application and treatment by this method is also limited, as water still needs to be pumped from the ground and the physical design favors gas phase mass transfer for volatile constituents.

Groundwater systems incorporating the use of subsurface recirculation show great potential for *in-situ* bioremediation. Configuration of the recirculating system is basic and centers on the use of a well screened over two intervals. The screened portions are hydraulically isolated from each other and water is pumped from one screened section and reinjected into the other. This technology minimizes pumping head required for water circulation and eliminates the regulatory issues associated with pumping ground water to the surface. Several applications of the dual screen recirculation system have been demonstrated using physical/chemical process for in-well contaminant removal. The biological applications of this technology have been limited (SBP, 1998). A recent full-scale demonstration by McCarty et al., 1998 incorporated the use of two recirculation wells for the aerobic cometabolic degradation of TCE in the presence of toluene. Using features of the site hydrogeology to separate the screens, water was pumped from the lower aquifer, amended with nutrients and injected back into the upper. Conversely, a second well 10 meters away pumped from the upper and discharged to the lower. The system performed very well, yet it was designed and constructed around unique geological conditions that may not be present on all sites.

The benefit of not removing water from the ground cannot be overemphasized when comparing remediation technologies. In the long term, pump and treat systems suffer from high operation costs (e.g. pumping costs, maintenance, discharge permits, etc). If treatment could occur *in-situ* removing the need for groundwater extraction, many of the long term operational costs could be eliminated. As such, recent attention has been directed toward the use of *in-situ* reactive walls or curtains. Conceptually derived by McMurty and Elton, 1985 and later expanded by Starr and Cherry, 1994, the remediation system is based upon the interception of a contaminant plume down gradient from its source with a permeable yet reactive barrier. The barrier is placed within the aquifer structure so that groundwater is contacted and reacted with the media as it moves through the treatment wall. Contaminant free water exits on the down gradient side of the wall. Treatment of small plumes is accomplished using a single treatment wall. Whereas,

interception and treatment of larger plume is achieved through a combination of multiple treatment walls and methods to control the subsurface groundwater flow. Starr and Cherry, 1994 later trademarked the combination of reactive walls and hydrological control as a "funnel-and-gate system".

The permeable barrier technique is applicable to a wide array of physical, chemical or biological treatment techniques. Operation of the system differs only by the reactive media chosen to construct the treatment wall. Construction media to support the adsorption of benzene from groundwater has been evaluated by Rael et al., 1995. Reduction and precipitation of chromium(IV) to insoluble hydroxides of chromium(III) by iron bearing solids has been investigated by Blowes et al., 1997. Biological reactive walls have also been proposed for reduction of sulfate and precipitation of metals in leachate migrating from mine tailings (Waybrant et al., 1998). Current developments in reactive treatment media have focussed on zero valent metals and their ability for abiotic reduction of chlorinated solvents (Matheson and Tratnyek, 1994; Roberts et al., 1996). The use of zero valent iron permeable barriers for the reduction of chlorinated solvents at full-scale has been reported by Puls and Powell, 1997, including one case for a waste mixture of chromium(IV) and trichloroethylene (TCE). Advances in reactive barrier media have led to the development of bi-metal systems for reduction. Incorporating a nickel plated zero valent iron media, installation of the deepest known permeable barrier began in late November 1996. The barrier was installed between the depths of 80 and 150 feet for the remediation of large TCE plume at Otis Air Force Base on Cape Cod, Massachusetts (Appleton, 1996). Table 3.1 presents a summary of field and pilot scale permeable barrier technologies currently in use. Clearly, the focus of permeable barrier application has been in the remediation of chlorinated solvents by zero valent iron reduction. Conversely, the application of biological based permeable barrier treatment regimes has primarily been limited to laboratory based column experiments. Full-scale treatment systems for biological treatment of pentachlorophenol-contaminated groundwater have been proposed. However, current field applications focus on technology demonstration at the pilot scale (Cole et al., 1998).

In an effort to develop a biological remediation strategy for the remediation of pentachlorophenol contaminated groundwater, an *in-situ* permeable barrier was designed and constructed. *In-situ* groundwater treatment is achieved using a large diameter well and a permeable biological reactor installed within a screened interval of the contaminated aquifer. The reactor is equipped with nutrient delivery and mixing systems for the support of a subsurface biological population. Environmental conditions are controlled from the surface and allow the operation of three unique (e.g. anaerobic, aerobic) treatment zones. Biodegradation of the aqueous phase contaminant occurs over the length of the reactor. Currently, the technology is in demonstration at the pilot scale at an active wood preserving facility in Eugene, Oregon. Results of the system operation and performance of the treatment process are described in the following paper.

Table 3.1 Summary of permeable barrier installations and treatment methods

Installation Location	Date	Target Pollutants	Treatment	Reference
USCG Base Elizabeth City, NC	June 1995	Solvents TCE (10mg/L), Metals Cr(VI)(10mg/L)	Fe^0 Reduction	(Puls and Powell, 1997)
Semi-Conductor Facility Mountain View, CA	Sept. 1995	Chlorinated Solvents *cis*-DCE (2 mg/L)	Fe^0 Reduction	(Puls and Powell, 1997)
Industrial Facility Belfast, N. Ireland	Dec. 1995	Chlorinated Solvents TCE (300 mg/L)	Fe^0 Reduction	(Puls and Powell, 1997)
Industrial Facility Coffeyville, KN	Jan. 1996	Chlorinated Solvents TCE (400 μg/L)	Fe^0 Reduction	(Puls and Powell, 1997)
Moffet Field Mountain View, CA	Apr. 1996	Solvents TCE (2 mg/L) *cis*-DCE (300 μg/L)	Fe^0 Reduction	(Sass et al., 1998)
Government Facility Lakewood, CO	Oct. 1996	Solvents VC (15 μg/L) TCE/DCE (700 μg/L)	Fe^0 Reduction	(Puls and Powell, 1997)
Otis Air Force Base Cape Cod, MA	Dec. 1996	Chlorinated Solvents TCE (100 μg/L)	Ni/Fe^0 Reduction	(Appleton, 1996)
Maintenance Facility Medford, OR	Fall 1997	Chlorinated Solvents *cis*-DCE	Fe^0 Reduction	(Romer and O'Hannesin, 1998)
Hill, Air Force Base Ogden, UT	ca. 1997	Chlorinated Solvents TCE	Fe^0 Reduction	(Wray and McFarland, 1998)
McFarland Cascade Eugene, OR	Dec. 1997	Pentachlorophenol	Biological	(Cole et al., 1998)
ACEL Chalk River, ON	Proj. 1998	Metals ^{90}Sr	Zeolite Adsorption	(Lee et al., 1998)

MATERIALS AND METHODS

Field based experiments on the *in-situ* biodegradation of PCP were conducted at the pilot scale using a custom designed permeable barrier reactor. The reactor assembly was constructed to fit within the casing of a large diameter well that was constructed in a PCP-contaminated aquifer at the L.D. McFarland facility in Eugene, Oregon. Major components of the reactor are described in the following sections; detailed equipment lists and shop drawings are included in Appendix B through Appendix F.

Sampling Procedure

Prior to the installation of the permeable barrier reactor, aqueous samples were collected from the aquifer structure using a variety of techniques. Early in the site characterization process, Teflon® bailers were used to collect samples and validate operation of the pneumatic sampling system designed for the permeable barrier reactor. All samples were collected within the screened interval of the well. The size

and storage volume of the 24" well casing prevented any type of purging procedure. The pneumatic sampling system consisted of a sample loop, two check valves placed in opposition, a three-way valve and a regulated low pressure gas source. The system was charged with nitrogen to a pressure of 25psig and lowered into the well. Once lowered to the desired depth, the system was depressurized to atmospheric conditions. The pressure of the water column in the well, now greater than the cracking pressure of the check valve, allowed the introduction of water into the sample loop. The sample loop was recharged with nitrogen. A two-way valve was throttled to allow for the displacement of gas and collection of the sample contained in the tubing loop. The liquid sample expelled by the nitrogen was collected in a 4 ml amber vial and sealed with a Teflon® faced screw cap. Samples were stored on ice for transport to the analytical laboratory.

Reactor System

The treatment unit was fabricated to design specifications by the engineering service shop at Oregon State University (Corvallis, OR). The reactor assembly was constructed with aluminum and stainless steel components of standard shapes and sizes. To minimize corrosion, all the aluminum components were coated and sealed with Teflon®. In finished dimensions, the reactor is 22" x 36" (diameter x height). Within the cylindrical shell, a rectangular section 36" x 15"x 18" (height x width x length) was created to contain the biological treatment processes. Vertical screen partitions were placed in the rectangular section to create an array of treatment and mixing zones. Screen surfaces were used on the inlet and outlet of the treatment area. Area outside the rectangular treatment cell was contained with solid sheet steel and created two crescent shaped spaces. The areas not occupied by the treatment cells were used to accommodate and protect sampling, nutrient supply, mixing and physical support systems.

The cylindrical unit is constructed of modular components, which allow for the creation of treatment and mixing zones. In its current configuration, the unit is assembled to operate with three biologically active zones. Growth within these zones is supported on ceramic saddles that possess both high surface area and hydraulic conductivity. Each zone is separated by one-inch vertical partitions that serve as nutrient supply and mixing areas. Nutrient addition consists of continuous low flow injection of a highly concentrated aqueous feed solution. Periodic agitation of the treatment zone influent is conducted by a gas lift mixing scheme. Inert gas or oxygen is used in the mixing regime depending on the desired environmental condition of the biological zone. All nutrient supply systems are isolated to allow for independent operation regardless of location within the reactor. Figure 3.1 shows the reactor in plan view while; Figure 3.2 presents a cross section of the unit.

Nutrient Control and Mixing System

Mixing and nutrient supply systems for the permeable barrier reactor operated in unison. In the base of each mixing zone, nutrient supply lines joined a horizontal diffuser, creating a nested tube assembly. The diffuser was constructed of slotted stainless steel tubing and extended the complete width of the mixing

zone. When supplied with a charge of compressed gas, nutrients and water were displaced from the diffuser assembly. Ejection of water and gas through the diffuser slots provided an opportunity for vigorous nutrient mixing and delivery. Vertical distribution of the mixture occurred by gas lift when, the buoyant gas bubbles rose upward through the open mixing zone. The diffuser slots were oriented toward the rigid screen boundary of the mixing and biologically active zone. The screen functioned as a baffle and helped to slow and break the upward flow of large gas bubbles from the diffuser assembly. Figure 3.3 depicts a schematic of the permeable barrier reactor nutrient supply and mixing system.

Operation and control of the mixing and nutrient supply system in the treatment zone A was independent from the tandem operation of treatment zones B and C. Electron donor was continuously pumped to the reactor mixing zones through 1/8" O.D. (3.2 mm) Teflon® tubing and two FMI QG-6 positive displacement pumps, Fluid Metering Inc. (Oyster Bay, NY). Two standard size gas cylinders and two-stage regulators were used to supply low pressure mixing gas to the diffuser assemblies. Mass flow of the mixing gases was controlled by two adjustable electric solenoid valves Cole-Parmer® (Vernon Hills, IL). The valve system used for mixing allowed control of both duration and frequency of activation. Over the course of the experiment, variations in mixing duration, frequency and location were evaluated.

Sampling System

Using the pneumatic sampling principle previously outlined, sample points were added to the permeable barrier reactor. Each collection point operates as an independent channel and allows the acquisition of discrete small volume samples. The sample points are attached to a control manifold and distributed in a logarithmic pattern that repeats at the mixing zone of each biologically active area. Two manifolds, each with 14 sample points are evenly distributed over the reactors height. Twelve sample points on each manifold are positioned along the centerline of the reactor. The remaining two sample points are placed along the periphery of the first mixing zone. Sample points along the centerline allow for the generation of longitudinal profiles while, those positioned in the mixing zone allow for characterization of one unique plane. Combination of sample points from the upper and lower manifolds allows for complete spatial and temporal characterization of biological removal processes.

Flow Cells

Two continuous flow cells were constructed and installed on recirculating sample loops to continuously monitor environmental conditions within the reactor's biological treatment zones. Water was pumped from the center of the anaerobic and aerobic treatment zones by a dual channel Masterflex® peristaltic pump, Cole-Parmer® (Vernon Hills, IL). To minimize solids uptake, sample inlets were screened with No.40 stainless steel mesh, McMaster-Carr Co. (Los Angles, CA). Samples were collected with PEEK 1/8" O.D. (3.2 mm) tubing Alltech Associates, Inc. (Deerfield, IL) to minimize oxygen diffusion. Teflon® 1/8" O.D. (3.2 mm) tubing was used for the gravity return line. The flow cells were custom designed and constructed

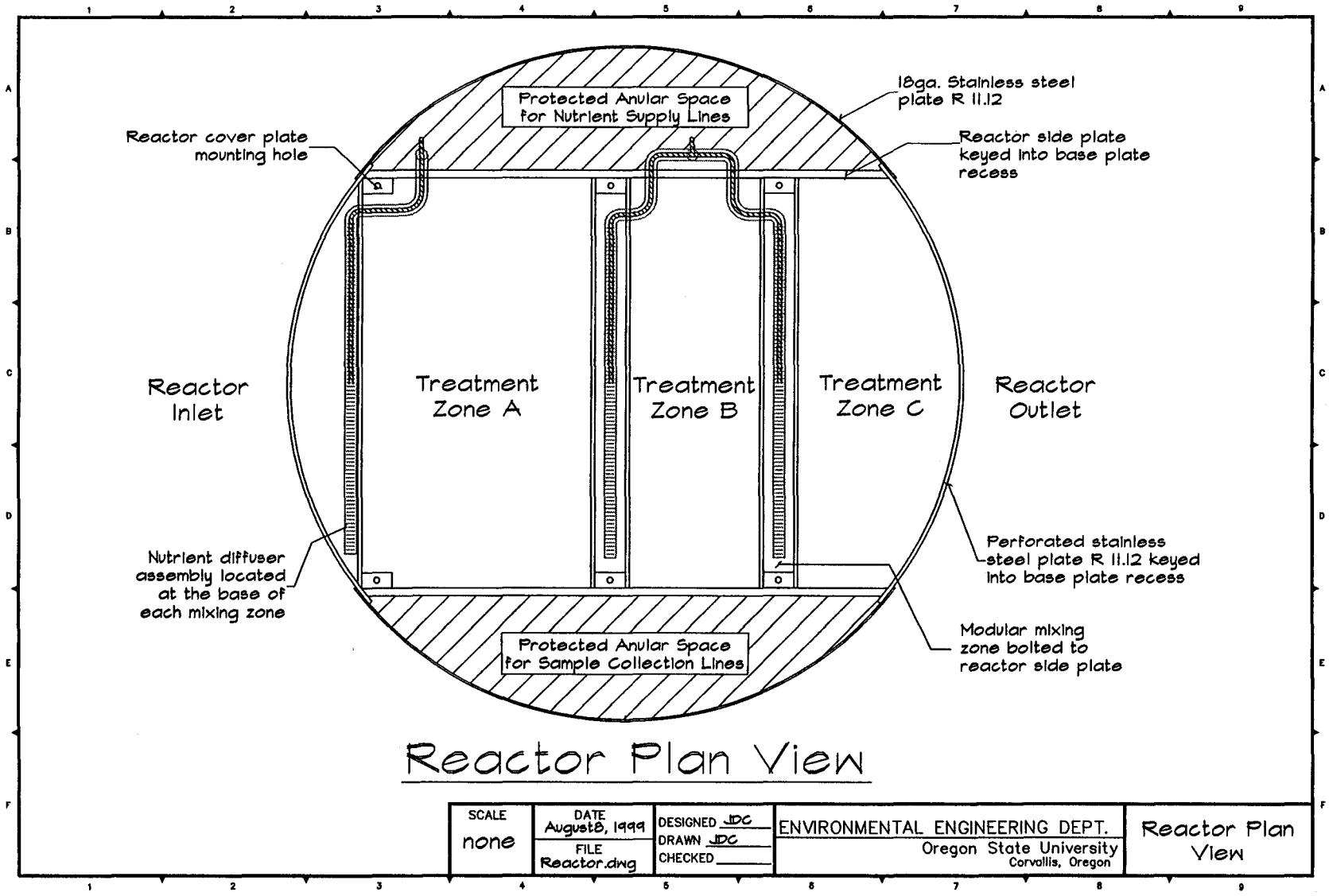

Figure 3.1 Permeable barrier reactor plan view

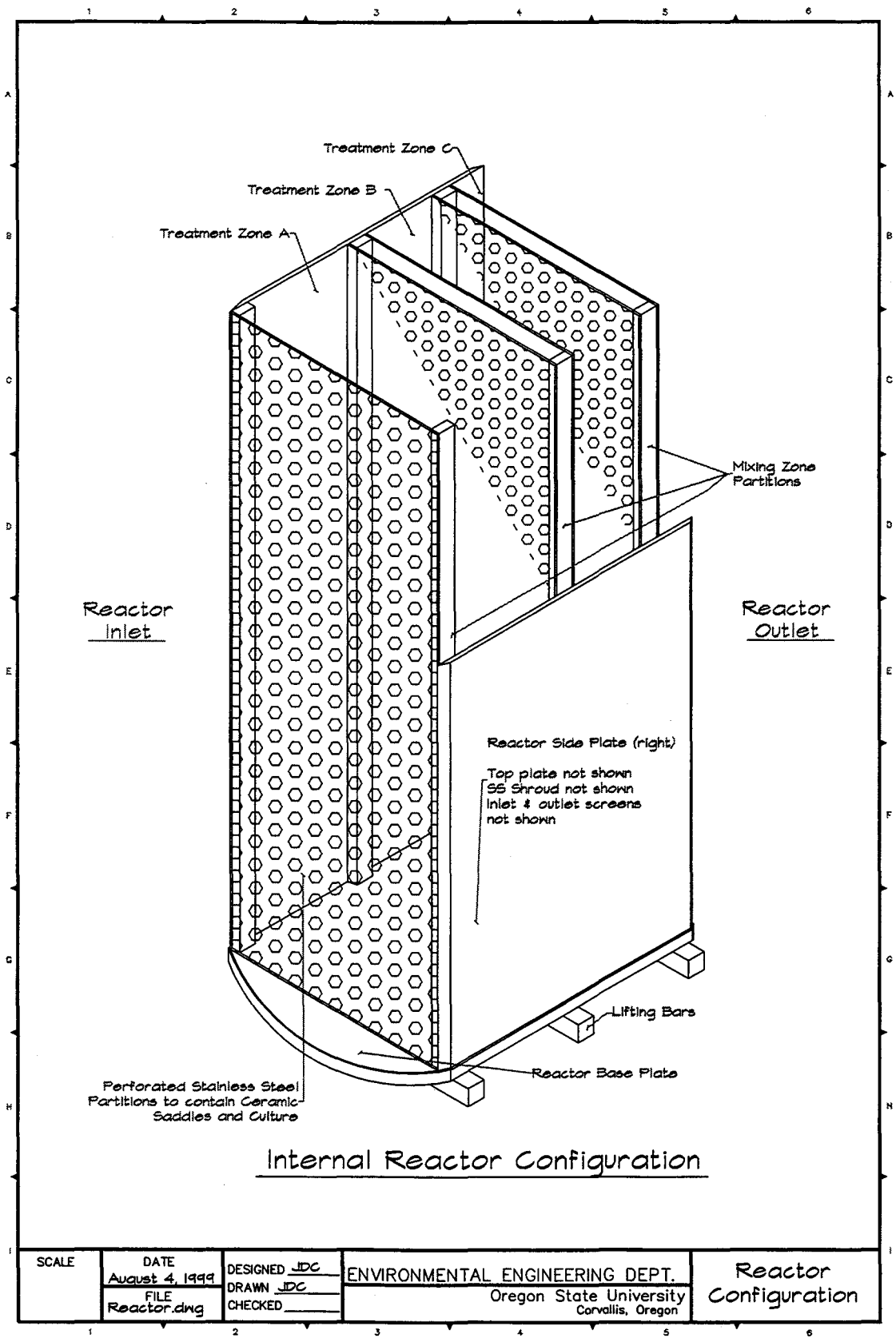

Figure 3.2 Permeable barrier reactor internal configuration

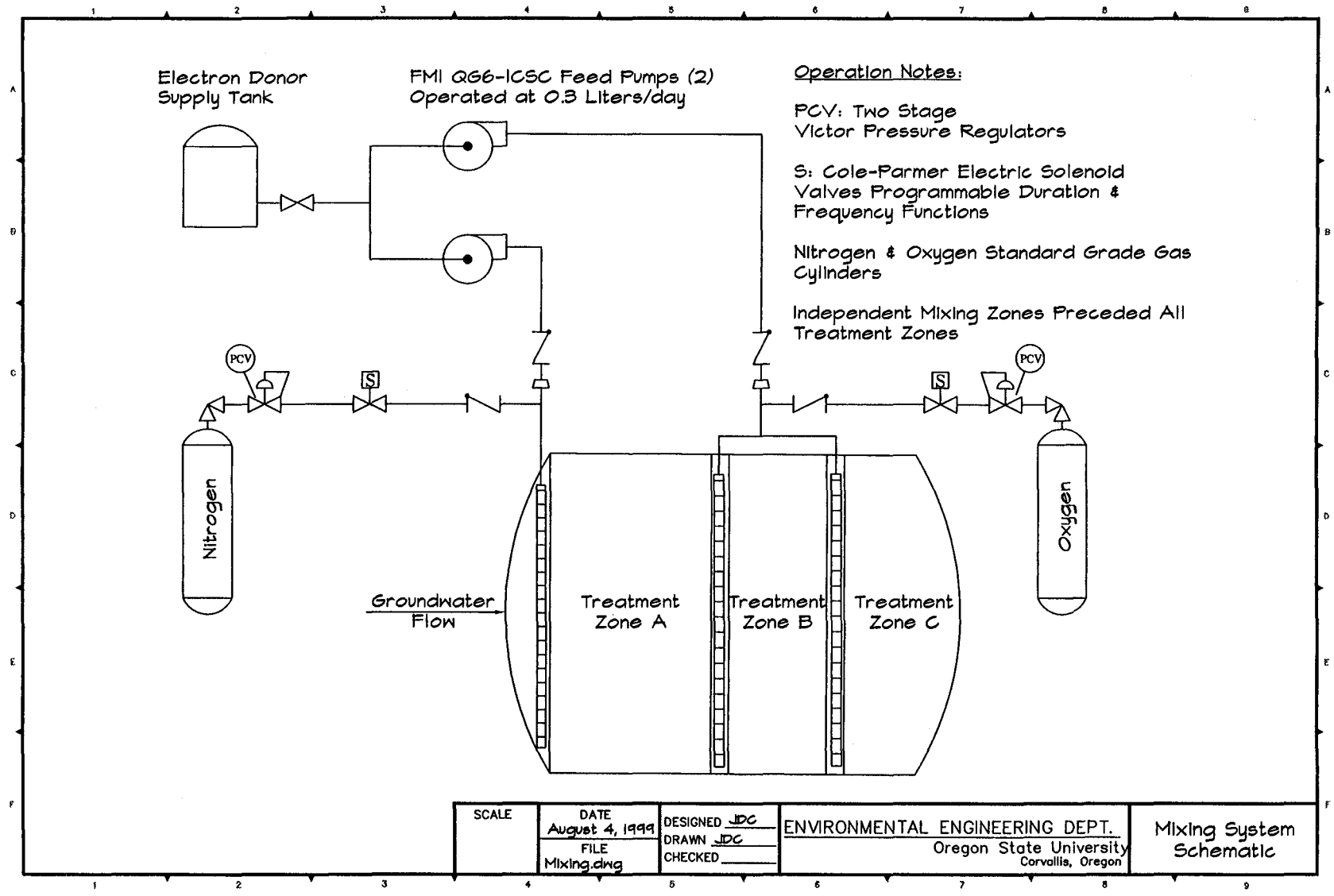

Figure 3.3 Reactor nutrient supply & mixing system schematic

with acrylic plastic. The finished internal volume of the flow cell measured 44 ml. Water was pumped from the two locations at 10 ml/minute to the base of each cell and flowed upward to the exit. The top of each cell was tapered to expedite the release of gas introduced by the pump. Low flow rates and equivalent mass removal and injection with the continuous loop design minimized preferential flow through the reactor. Both cells were completely mixed with magnetic plate assemblies and Teflon® coated stir bars. Spacers below the flow cells helped to minimize heat transfer from the stir plate.

The flow cells were designed to allow the use of three standard sized electrodes. Oxidation/reduction potential (E_H) and pH were measured real time in each cell using a pH combination glass body electrode Cole-Parmer® (Vernon Hills, IL) and a platinum E_H half cell, (Analytical Sensors, Inc. OR100031 BN). The combination pH electrode served as a common reference (Ag/AgCl gel) for each cell. The probes were routinely cleaned and calibrated in accordance with the manufacturers specifications. A custom interface was designed to handle the electrode signals in each flow cell. Type T copper-constantan thermocouples, Cole-Parmer® (Vernon Hills, IL), were used to monitor temperature differences between the groundwater system and the continuous flow cells. A Campbell Scientific 21X data logger (Logan, UT) was used for signal interpretation and data storage. On regular intervals, data was manually transferred to a portable computer and interpreted. Flow cell operation and instrumentation is summarized in Figure 3.4.

Inoculum

Consortia used in the study were harvested from a municipal wastewater treatment facility in Corvallis, Oregon. The anaerobic culture originated as a combination of return secondary sludge from an activated sludge system and supernatant from the anaerobic sludge digester. Liquid cell suspensions from each environment were mixed 50/50 on a volume basis and dispensed into ten five-gallon plastic buckets, which were filled, with No. 2 ceramic saddles, Jagger Products (Dallas, TX). The buckets were tightly sealed with rubber lined plastic lids. Five buckets were maintained aerobic by the injection of compressed air. A water filled gas trap was installed in the lids of the remaining buckets in and effort to promote anaerobic conditions. For a period of six months, the buckets were stored together prior to use in the field. During this time, no supplemental carbon source or electron donor was added. Furthermore, there was no attempt to acclimate the organisms to PCP.

Chemical Sources

Individual components of the imitation vanilla flavoring were obtained from Acros Chemical Co. (Pittsburgh, PA). All were reagent grade and possessed purity greater than 99%. Other chemicals consumed over the course of the experiment were obtained from Mallinckrodt Co., (Paris, KY) or EM Science, (Cherry Hill, NJ). Chlorophenol analytical standards were obtained from Ultra Scientific Inc., (North Kingston, RI).

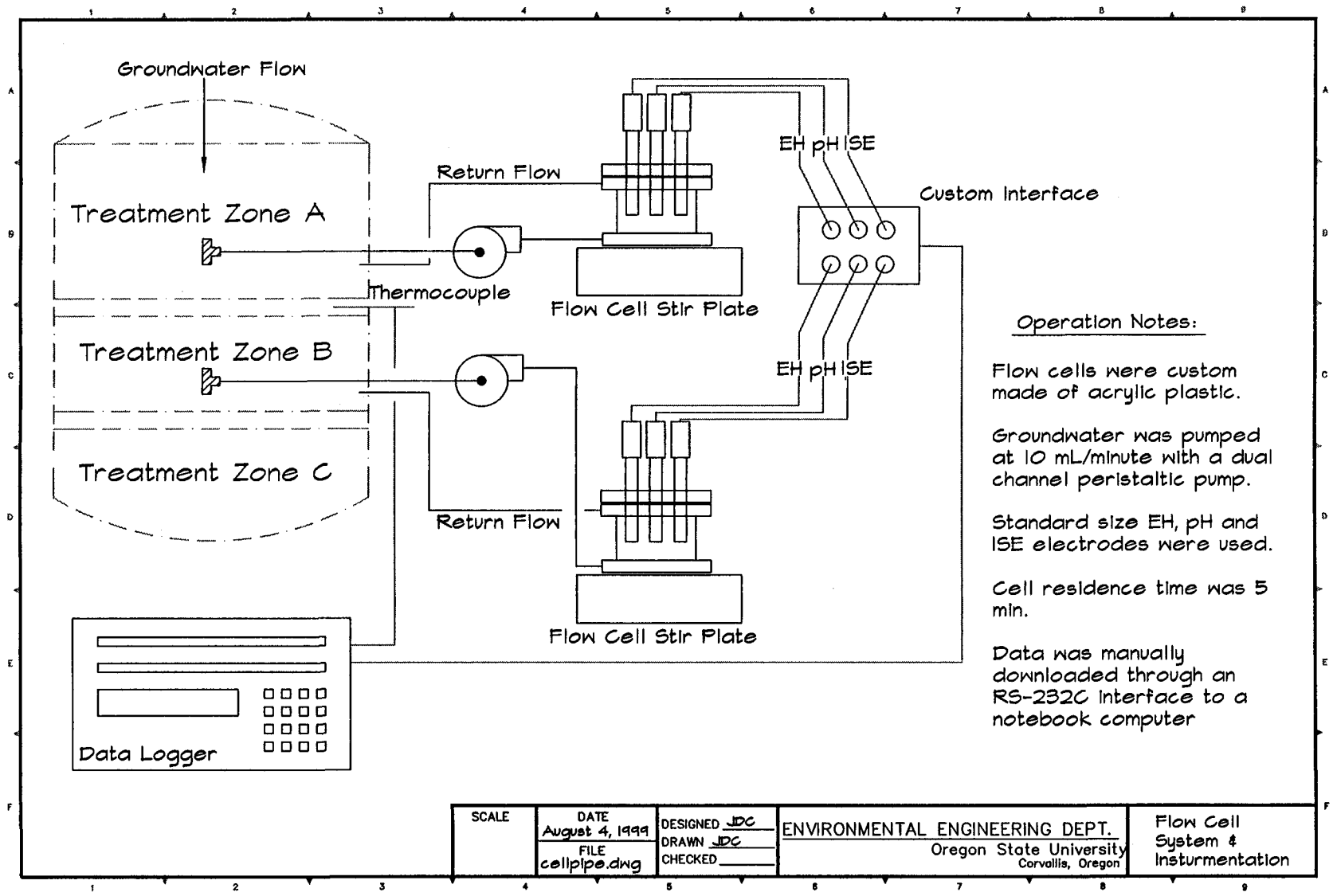

Figure 3.4 Flow cell piping and instrumentation schematic

Analytical Procedures

Aqueous samples collected from the well and reactor were analyzed for chlorophenol concentration by capillary gas chromatography. Samples were acetylated and extracted into hexane using a modification of the method developed by Voss et al., 1980 and NCASI, 1981. Extractions were conducted as follows: 500 µl of a solution containing 30.4 g/L K_2CO_3 and 250 µg/L 2,4,6 tribromophenol (an internal standard) was combined with a 100 µl aqueous sample in a disposable glass culture tube with a Teflon® faced cap. 100 µl of acetic anhydride was added and the tube was mechanically shaken for 20 minutes. 1 ml of chromatographic grade hexane was added and the tube was shaken for an additional 20 minutes. Hexane was removed from the tube and transferred to a 2 ml amber glass vial. The vial was sealed with a Viton® faced crimp cap. Vials were immediately loaded for analysis by capillary gas chromatography.

Chlorophenols were quantified on a Hewlett Packard 6890 gas chromatograph with a ^{63}Ni electron capture detector (ECD). Automated 1 µl injections were made on the inlet, which was operated, in a splitless configuration. Separation of chlorophenol congeners was accomplished on a DB-5 fused silica capillary column (30m x 320µm I.D. x 0.25µm film; J & W Scientific, Folsom, CA). Helium provided at 35 cm/s served as the column carrier gas. A 95/5 blend of argon/methane at 75 ml/min was used for detector make-up. The instrument was operated as follows: initial temperature of 45°C was held for 2 minutes; the temperature was then increased 25°C/min to 140°C and held for 5 minutes; the oven was then increased 5°C/min to 250°C where it was held for 10 minutes. Anion measurement of selected samples was accomplished with a Dionex 2000I ion chromatograph.

Site Description

The permeable barrier reactor was selected for demonstration at an active wood preserving facility in Eugene, Oregon. The facility began operation in the-mid 1950s and applied PCP in a medium aromatic treating oil to telephone poles. Several process variations over the years occurred but without change in treatment chemicals. Operational practices and several accidental spills resulted in contamination of the underlying aquifer with PCP and its carrier oil. Subsurface remedial action measures center around four groundwater recovery wells. Groundwater contamination off site has been mitigated by the reversal of local groundwater flow. Free oil is removed from the water surface by skim pumps when required. Water removed from the aquifer is treated by granular activated carbon while, recovered oil is returned to the process.

Several comprehensive geologic studies have been conducted at the facility since the identification of subsurface contamination. Soil borings and well construction logs have identified the aquifer on site is a shallow semi-confined structure comprised of two major geologic units. The upper geologic unit averages 10 feet in thickness and is characterized as a dense yet, permeable clay formation. Underlying the clay and ranging in thickness, are well-sorted sands and gravel. Historical measurements reveled groundwater

elevations vary seasonally and range from 5 to 15 feet (1.5 to 4.6 meters) below ground surface (bgs). Figure 3.5 summarizes the boring and well locations at the L.D. McFarland facility (RETEC, 1994).

The reactor test site was chosen between two of the site's groundwater recovery wells. The location selected offered complete hydraulic control and excellent accessibility. From a geological perspective, the location of demonstration was ideal, as the lower confining layer of the aquifer was identified at a minimum distance bgs. Cross sections constructed from boring logs (Figure 3.6) estimated the depth of the lower confining layer at 25 feet (7.6 m) bgs. In March of 1996, a 24" (0.61 m) diameter well was installed in a protected concrete vault on site. The well was constructed using a cable tool rig and was drilled to a depth of 25 feet (7.6 m). A section of wire wrapped stainless steel screen was placed in the gravel section of the aquifer from of 18 to 21 feet bgs. Carbon steel was used in to construct the sump and riser. The well head was protected in a locking flush-mount concrete vault. Details of well construction and lithology of geologic units encountered are summarized in the drilling log shown in Figure 3.7.

RESULTS

The development and demonstration of an *in-situ* biological treatment technology at the L.D. McFarland facility has been in progress since late 1996. During the pilot demonstration, several operational conditions were imposed on the permeable barrier reactor. Process and experimental changes are summarized in Table 3.2. Where noted, imitation vanilla flavoring served as the electron donor for the system. Roman numerals and vertical partitions provide a universal legend for the figures and represent the process changes evaluated and their relative duration. Chlorophenols present in MW 96-1, the location of the pilot scale demonstration reactor system, are summarized in Figure 3.8. Concentration of all species quantified is reported in mg/L on the left axis; water elevation, in feet, referenced to mean sea level (MSL) is presented on the right axis. Environmental conditions of the reactor system expressed by apparent E_H and pH are presented with time in Figure 3.9. Apparent E_H, referenced to the standard hydrogen electrode, is presented in millivolts (mV) on the left axis; pH measured in the treatment zones is presented on the left axis. Chlorophenol concentrations as function of time during each experimental phase are presented in Figure 3.10 through Figure 3.13. In all figures, PCP concentration is presented on the on the left axis; the right axis corresponds to all other species quantified.

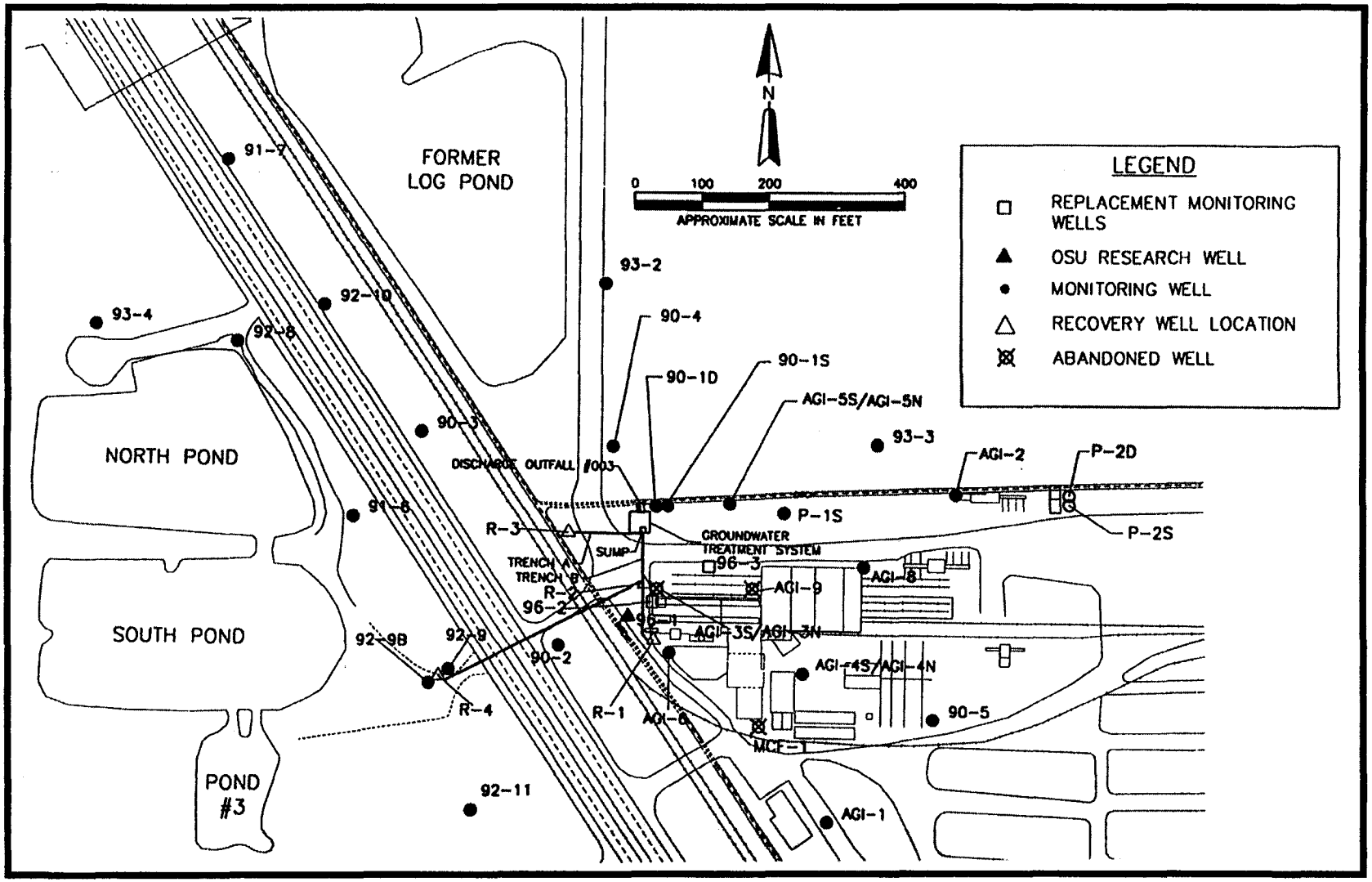

Figure 3.5 Well and boring locations at the L.D. McFarland Facility

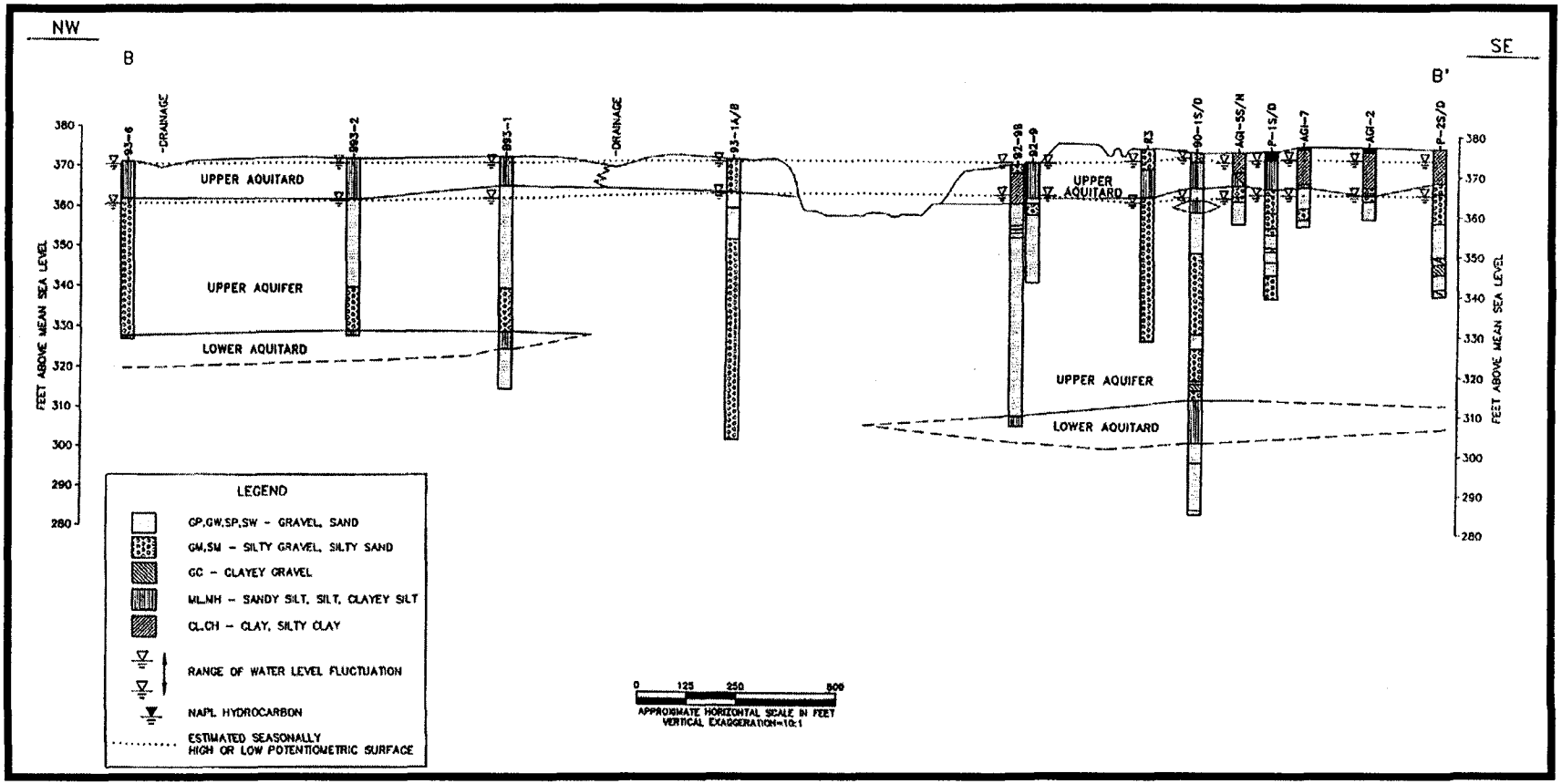

Figure 3.6 Geologic cross-section at the L.D. McFarland Facility

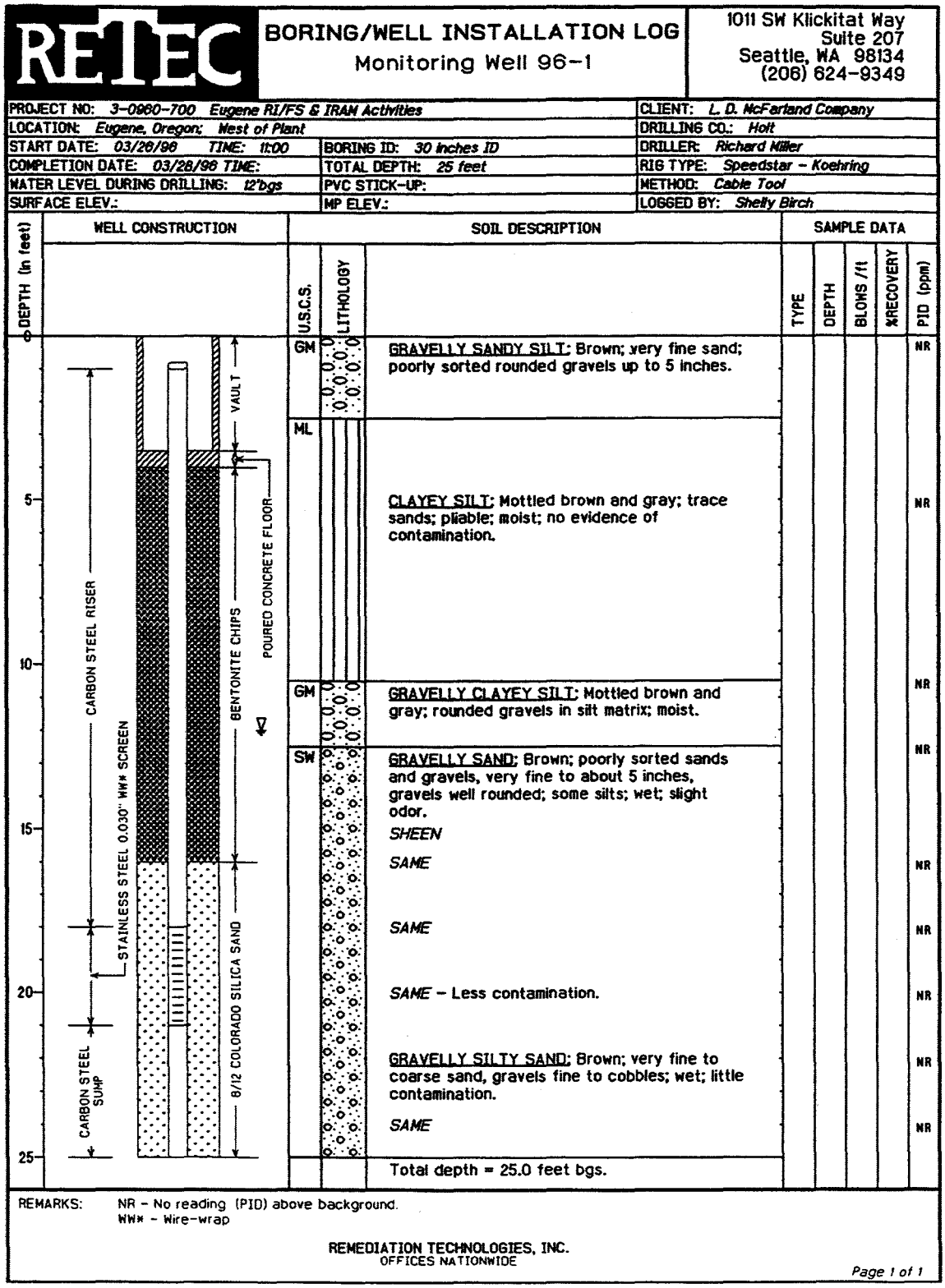

Figure 3.7 Reactor well log and construction details

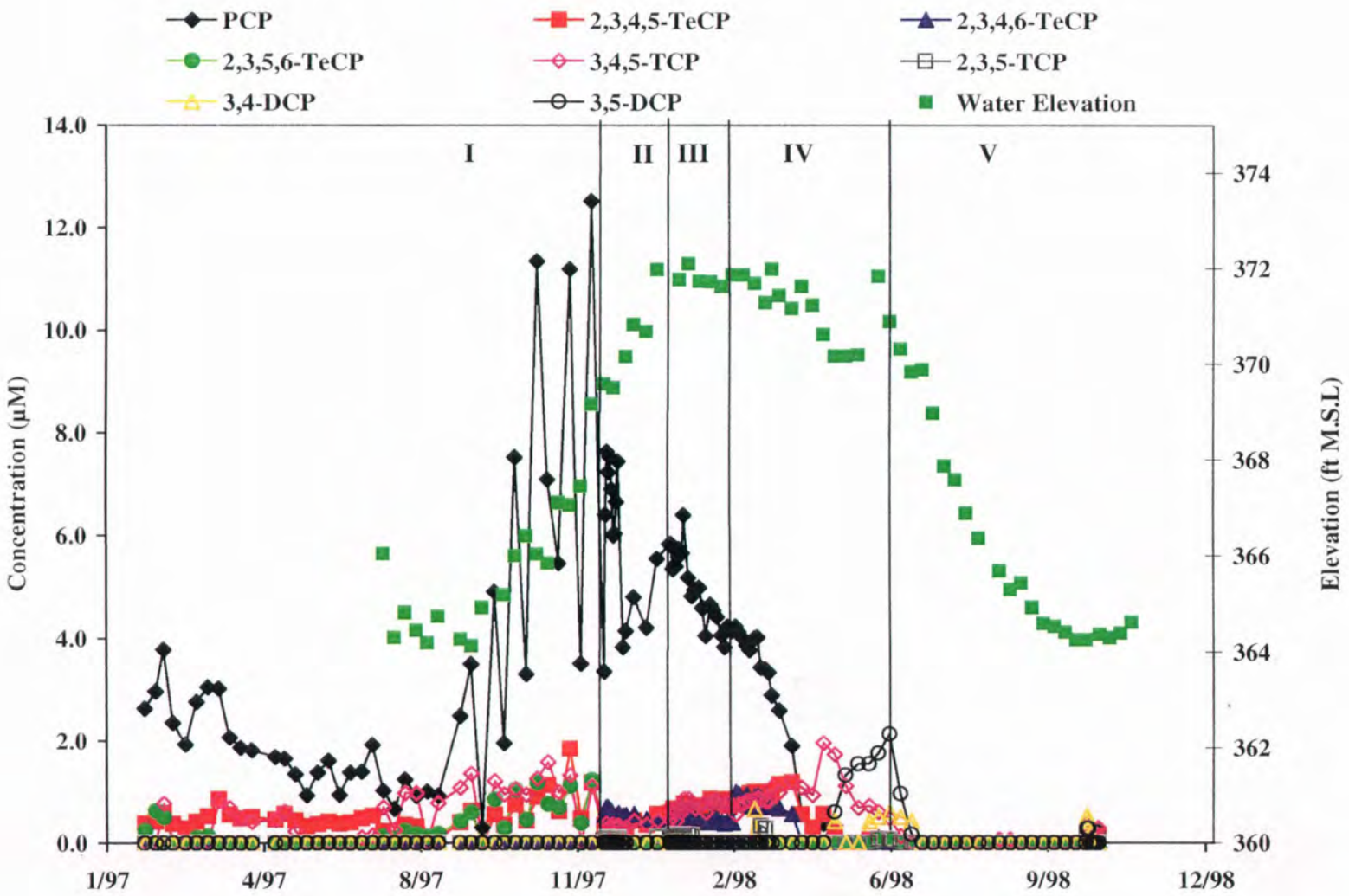

Figure 3.8 Chlorophenols observed in an in-situ permeable barrier reactor

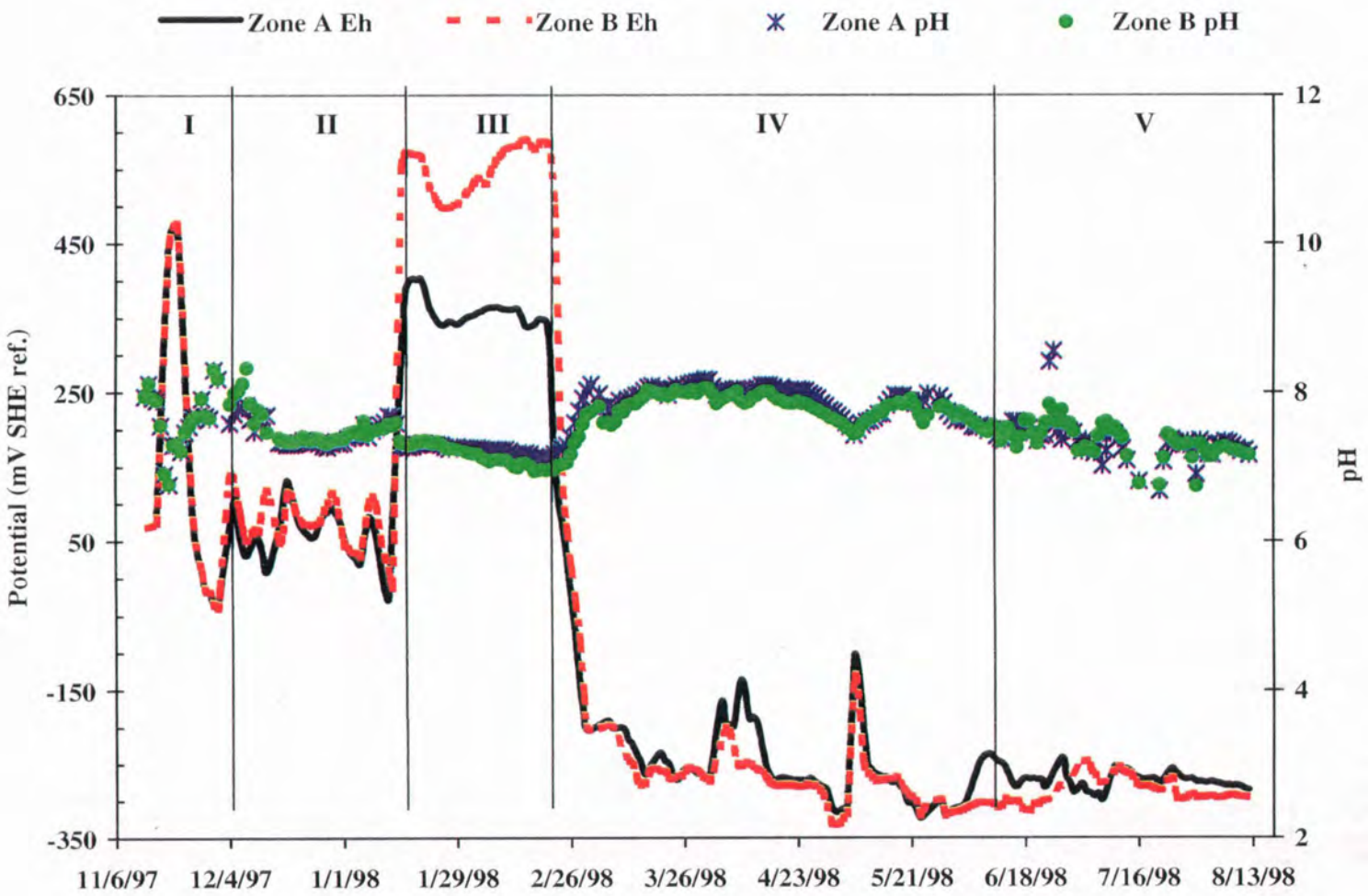

Figure 3.9 Environmental conditions observed in an in-situ permeable barrier reactor

Table 3.2 Summary of experimental conditions evaluated

Phase	Process Conditions	Mixing Conditions	Start	Stop
I	No inoculum, electron donor or acceptor	No mixing	2/13/97	12/3/97
II	Inoculum, no electron donor or acceptor	No mixing	12/3/97	1/15/98
III	Inoculum, electron donor & acceptor *Mixed Anaerobic / Aerobic Conditions*	Nitrogen 2 sec./15 min Oxygen 2 sec./15 min	1/15/98	2/27/98
IV	Inoculum, electron donor & acceptor *Anaerobic Conditions Prevalent*	Nitrogen < 1sec./15 min Oxygen < 1sec./15 min	2/27/98	6/6/98
V	Inoculum, electron donor reduced 50%*Anaerobic Conditions Prevalent*	Nitrogen < 1sec./15 min Oxygen < 1sec./15 min	6/6/98	12/30/98

Phase I

Background data collection was the focus of phase I efforts in the demonstration study. Beginning in February of 1997, weekly samples were collected and analyzed for chlorophenols. Figure 3.10 presents chlorophenol concentrations observed during site characterization as a function of time. Fluctuations in PCP concentrations and a decreasing trend with increasing time reveled a direct correlation of PCP loading rates and water elevation in the unconfined aquifer (Figure 3.8-I). PCP groundwater concentrations at the site generally decreased through the dry summer months and increased during the wet winter months. PCP behavior in the reactor demonstration well was similar to concentrations observed at other monitoring wells on site. Despite the seasonally induced decrease observed in PCP concentration, 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and 3,4,5-trichlorophenol (3,4,5-TCP) present in the system remained relatively constant with time. The erratic response of the PCP measured through the fall months was mirrored in the response of the lower order chlorinated phenols. The increasing concentration trend observed in all species shown followed the seasonal increase in static water elevation. Analysis of historical site data accounted for the presence of the lower order chlorinated phenols as a circumstance of technical PCP formulation rather than products of biological transformation.

Phase II

During the first week of December 1997, the reactor assembly was removed, inoculated and returned to the well in the absence of electron donor and acceptor. Seasonal rains continued to influence contaminant loading as static water elevation increased more than two feet during phase II of the study (Figure 3.8-II). Chlorophenols measured in the permeable barrier reactor as a function of time are

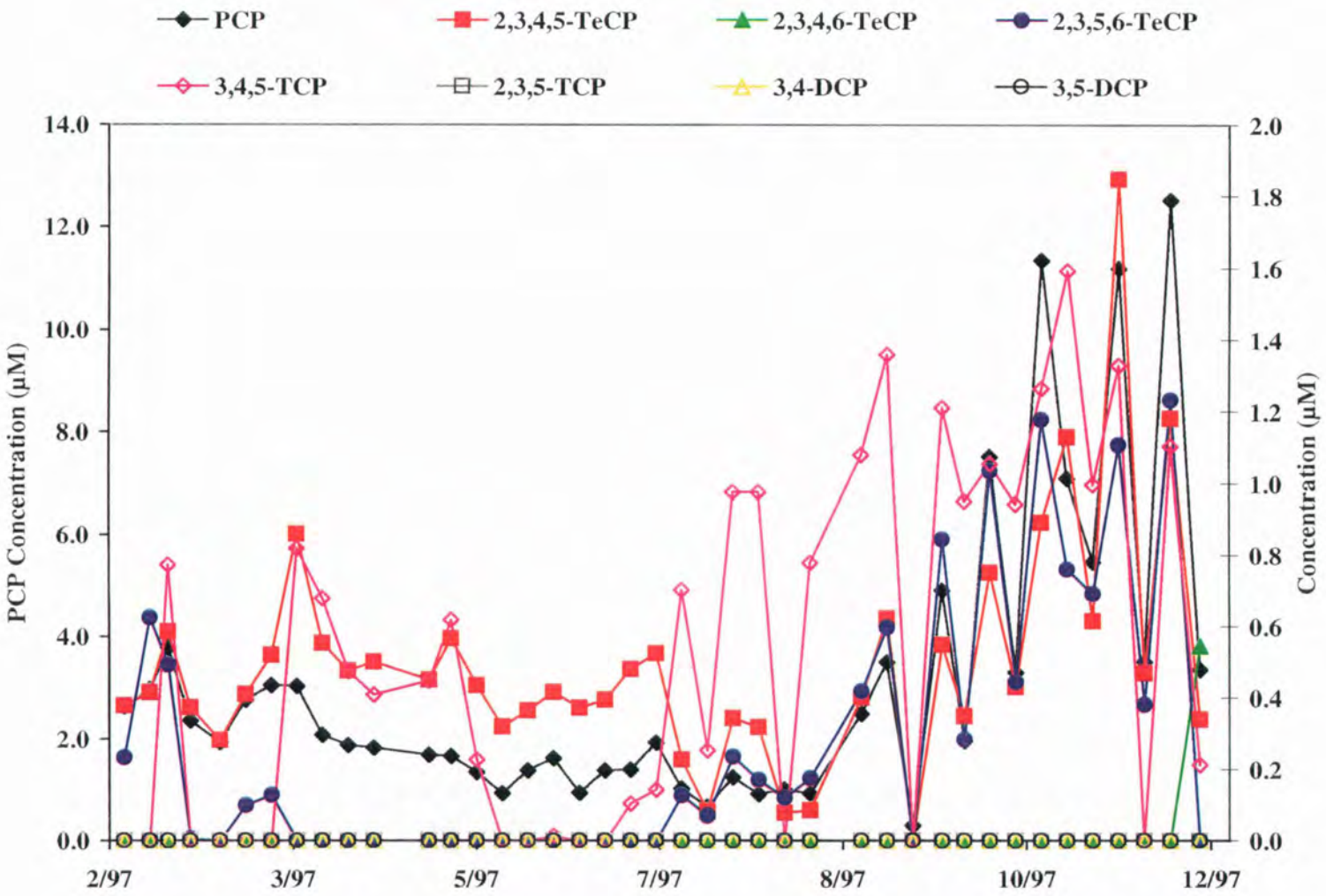

Figure 3.10 Chlorophenols observed during site characterization phase I

presented in Figure 3.11. PCP concentrations increased and nearly doubled early in phase II. However, with increasing time, PCP concentrations in the reactor decreased and stabilized. Despite the observed variability in PCP concentrations, lower order chlorinated phenols, present prior to inoculation, remained stable. Concentration profiles of all chlorophenols observed in the reactor as a function of time were similar and differed only in magnitude of change; variation in chlorophenol concentrations was consistent with field variability. This trend is shown during the transient PCP concentration peak by the prominent dip observed in all chlorophenols identified in the reactor system. Apparent E_H and pH data collected from the two biologically active zones within the reactor indicated no major changes in the overall environmental conditions (Figure 3.9-II). In the absence of electron donor addition, there was no evidence to support the biotransformation of PCP in the reactor system.

Phase III

In early January 1998, injection of the electron donor, imitation vanilla flavoring, began. Concurrently, the reactor assembly was mixed with nitrogen and oxygen gas lifts for a period of two seconds every fifteen minutes. The anaerobic treatment zone (A) received nitrogen; oxygen was used to mix the aerobic zones (B and C). Oxygen supply to treatment zones B and C was intended to create aerobic conditions that would allow for faster transformation rates of dechlorinated intermediates produced by anaerobic PCP degradation. The static water elevation of MW 96-1 remained constant during phase III (Figure 3.8-III). Reactor response to oxygen addition was immediate and resulted in a rapid increase in apparent E_H in both the anaerobic and aerobic treatment zones (Figure 3.9-III). At the onset of aerobic conditions, system pH measurements decreased approximately 0.3 units in both treatment zones. Aerobic conditions prevailed in the reactor for approximately one month. Figure 3.12 summarizes the response of PCP and lower order chlorinated compounds during phase III. Generally, concentrations of the chlorinated phenols showed no appreciable change during phase III. A gradual decrease in PCP and minor increases in 2,3,4,5-TeCP and 3,4,5-TCP concentrations was observed with increasing time. The behavior of 2,3,4,6-TeCP was an exception; its concentration doubled over the last three days of the experimental phase. Increasing concentrations of tetrachlorophenols quantified in the system indicated the potential for biological transformations. However, the environmental conditions present were not favorable for reductive biological processes.

Phase IV

Oxygen supply to the reactor during phase III created an environment that was considered inhibitory to PCP reductive dechlorination (Figure 3.9-III). The apparent E_H during phase III was greater than –300mV. Therefore, in an effort to preserve the concept of sequential anaerobic/aerobic environments and ensure PCP dechlorination, the duration of oxygen addition per mixing cycle was decreased. Regardless of oxidant addition to the reactor system, the response to the change in mixing was immediate. The apparent E_H in both treatment zones fell rapidly before eventually stabilizing near –270 mV (Figure 3.9-IV).

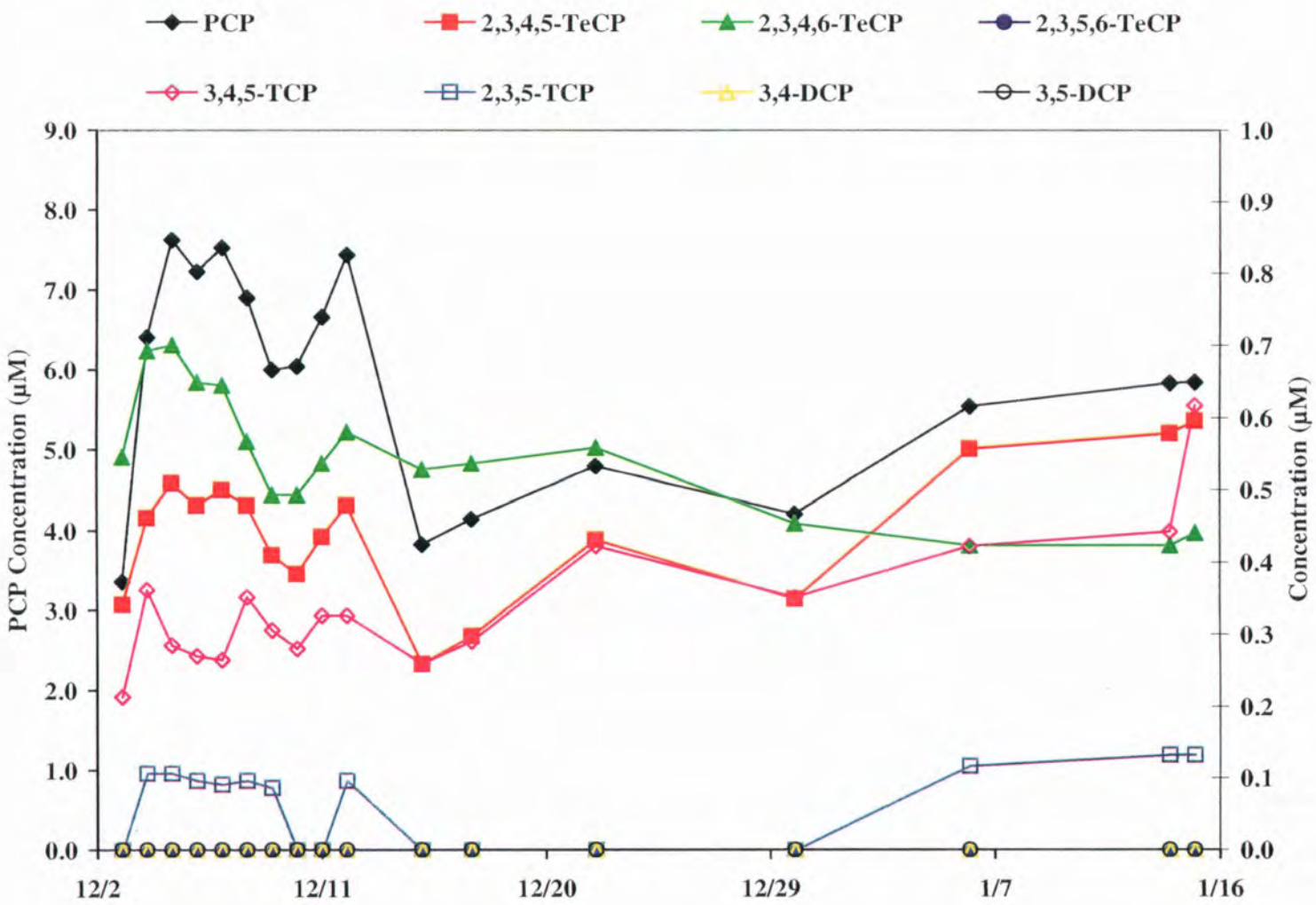

Figure 3.11 Chlorophenols observed during phase II

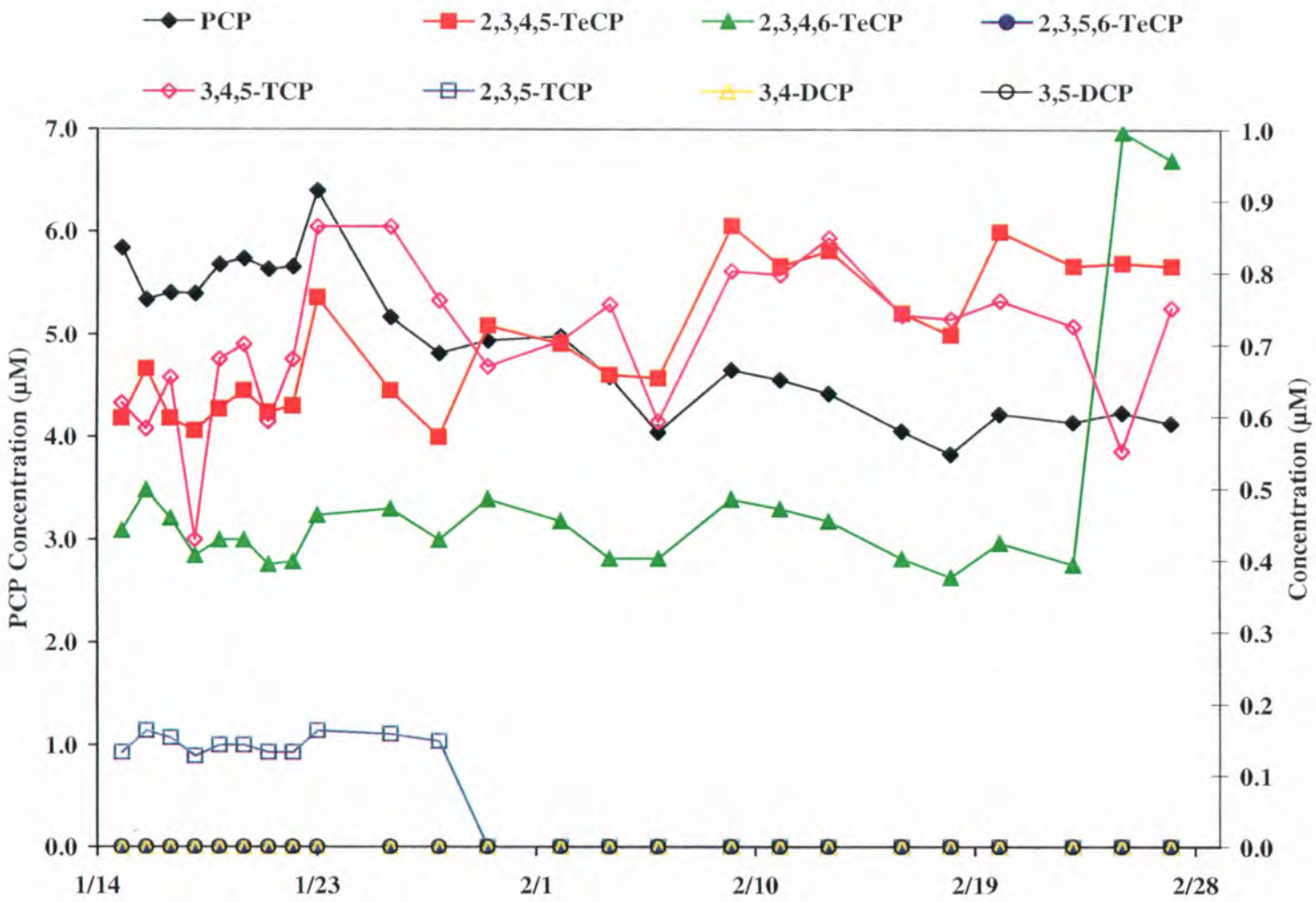

Figure 3.12 Chlorophenols observed during phase III

Reduction of oxidant to the reactor system also initiated an increase in pH that was observed in both treatment zones. Concurrent with the decline in the system apparent E_H, production of dechlorinated intermediates was observed (Figure 3.13). PCP dechlorination proceeded by initial dechlorination at the *ortho* position. Dechlorination at the *ortho* position to form 2,3,4,5-TeCP was immediate following the change in experimental conditions. With time, concentrations of PCP decreased while 2,3,4,5-TeCP increased. Production of 2,3,4,5-TeCP was transient and removal proceeded through a second *ortho* dechlorination producing 3,4,5-TCP. Concentrations of 3,4,5-TCP increased with time while 2,3,4,5-TeCP decreased to levels below detection. Removal of 3,4,5-TCP was observed by the production of both 3,5-dichlorophenol (3,5-DCP) and 3,4-dichlorophenol (3,4-DCP). Removal of both 3,4-DCP and 3,5-DCP was observed however, products of their degradation were not quantified. Complete removal of PCP under anaerobic conditions was observed in the permeable barrier reactor (Figure 3.8-IV and Figure 3.13).

DISCUSSION

The diverse range of environmental and hydrogeological conditions present at the McFarland site presented some unique challenges in the development of this technology. The relatively simple geologic formations on site and shallow contamination were offset by complex interactions between fluctuating water levels and inconsistent PCP loading rates. Initial site characterization was paramount in discerning the correlation between fluctuating PCP concentrations and groundwater elevation. As such, field experiments proceeded only after the collection of adequate background data.

Pentachlorophenol Loading

The erratic nature of PCP loading during phase I of the study may be due to several factors. PCP was released to the aquifer structure by way of the treating oil used in the process. The oil is a light non-aqueous phase liquid (LNAPL) and is distributed vertically in the aquifer structure as a function of the static water elevation. To date, recovery of the free LNAPL continues in the aquifer structure. Years of cyclic variation in water table elevation have likely formed a smear zone of PCP and oil in the surface clay layer. The layer of clay varies in thickness across the site. Drill logs of the reactor location approximate the thickness at 12 feet. Traditionally, the clay layer is saturated during the winter months and dry during the summer. As such, oil trapped in the clay structure only allows PCP dissolution during the periods marked by high water elevations. Generally, the highest PCP loading rates are associated with seasonal increases in static water levels (Figure 3.8).

Data presented in Figure 3.10 depict transient increases in PCP concentrations brought on by rapid increases in aquifer water table elevation. In the aquifer structure, the increase in water levels translates to a larger cross sectional area for the groundwater to contact residual LNAPL and PCP. Infiltration and

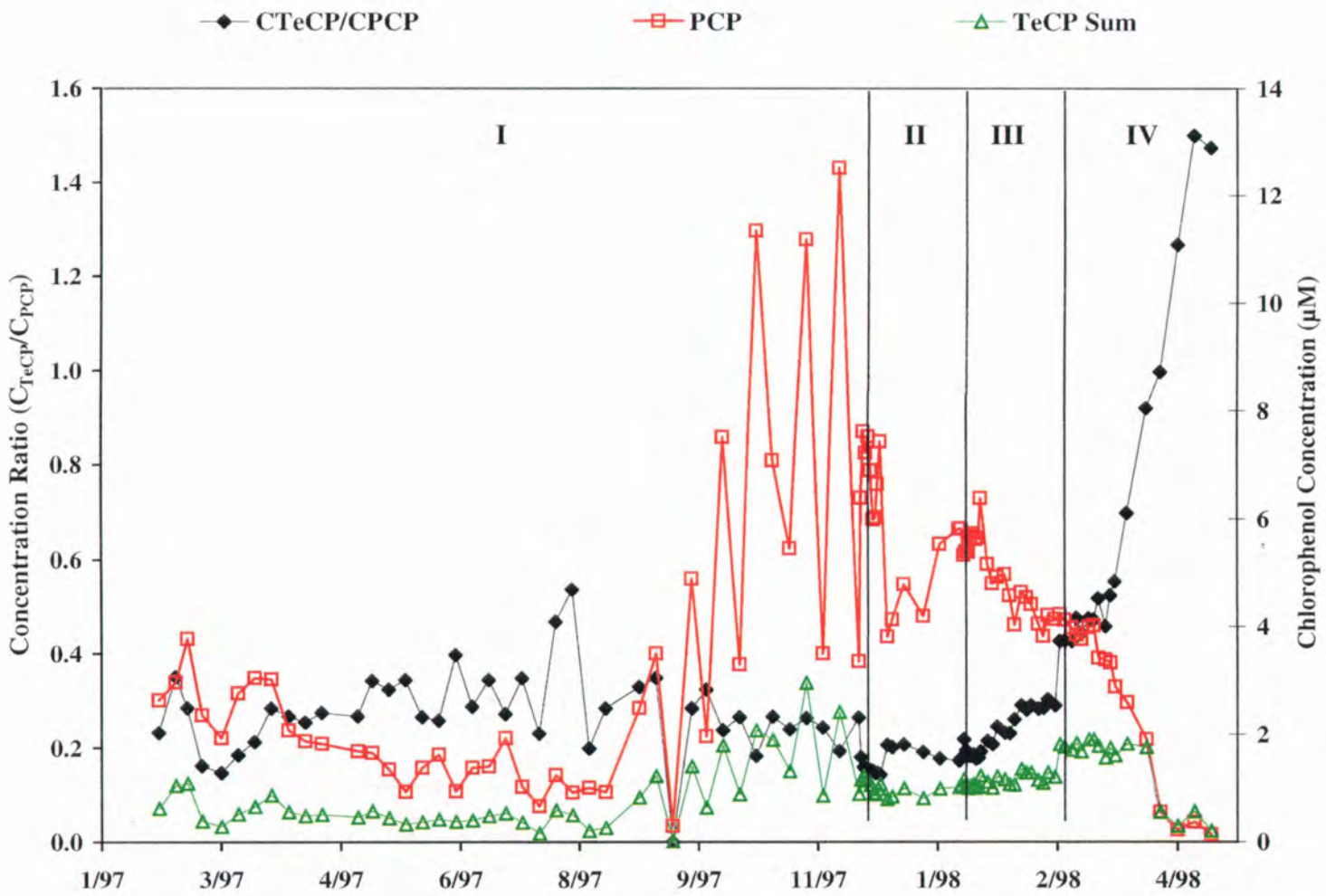

Figure 3.13 Concentration ratio of TeCP/PCP observed during phases I-IV

percolation of surface water to the aquifer likely contribute to the observed PCP concentration increases. Through the fall months of 1997, the tetrachlorophenols present follow the erratic pattern of PCP concentrations quite well. Despite shifting concentrations of PCP and lower order chlorinated phenols during phases I and II, the ratio of total tetrachlorophenol concentration to PCP remained virtually unchanged (Figure 3.14). Since the LNAPL is predominately composed of PCP, the behavior of PCP in the system reflects its concentration dominance over lower order chlorinated phenolic compounds.

Pentachlorophenol Degradation

In an effort to establish a culture capable of PCP dechlorination, environmental conditions within the permeable barrier reactor were manipulated. Following reactor inoculation, perturbations were made in a stepwise manner to discern the operational scenario that provided the most effective environmental conditions for *in-situ* PCP degradation. In phase II evaluation of the reactor reveled that cells unacclimated to PCP were unable to dechlorinate PCP by reductive processes. The period of acclimation to subsurface conditions in the absence of electron donor and electron acceptor addition showed little promise for PCP reductive dechlorination (Figure 3.11). PCP concentrations during phase II increased as a result of water table changes rather than decreased due to biological processes. The stable ratio of total tetrachlorophenols to PCP (Figure 3.14) through phase I and II further supports the link between chlorophenol loading rate and groundwater elevation. Lower order chlorophenols showed behavior similar to PCP concentrations measured. Data collected in the absence of electron donor and acceptor addition further reveled that PCP losses from the system due to biomass sorption were insignificant. If sorption to solids were in fact a major pathway of removal, suppression of PCP concentrations would have been expected following reactor inoculation. Conversely, the opposite effect, increasing PCP concentrations was observed.

In the conceptual design of this treatment strategy, degradation of PCP under sequential anaerobic and aerobic environments in a plug flow regime was proposed. To fulfill this concept, the reactor system was physically designed to provide three biological treatment zones. Disproportionate in size, treatment zone A was slightly larger in volume than the summation of the volume occupied by zones B and C. At the expense of operational flexibility, nutrient and mixing gas supply to treatment zones B and C were combined to reduce system hardware. Provisions to manipulate the environmental conditions in each of these treatment zones were based solely on regulation of the electron donor and acceptors pair supplied.

Addition of electron donor and acceptor to the reactor in phase III had an immediate effect on the environmental conditions in the permeable barrier reactor. Prior to the addition, apparent E_H measurements in both flow cells were nearly identical (Figure 3.9). Oxygen addition to the rear treatment zones (B and C) however, had a profound effect on the environmental conditions reactor wide. Although apparent E_H measurements were somewhat lower than conditions in the aerobic zones (ca. 200 mV), apparent E_H measurements in the anaerobic treatment zone were still significantly above the approximate baseline

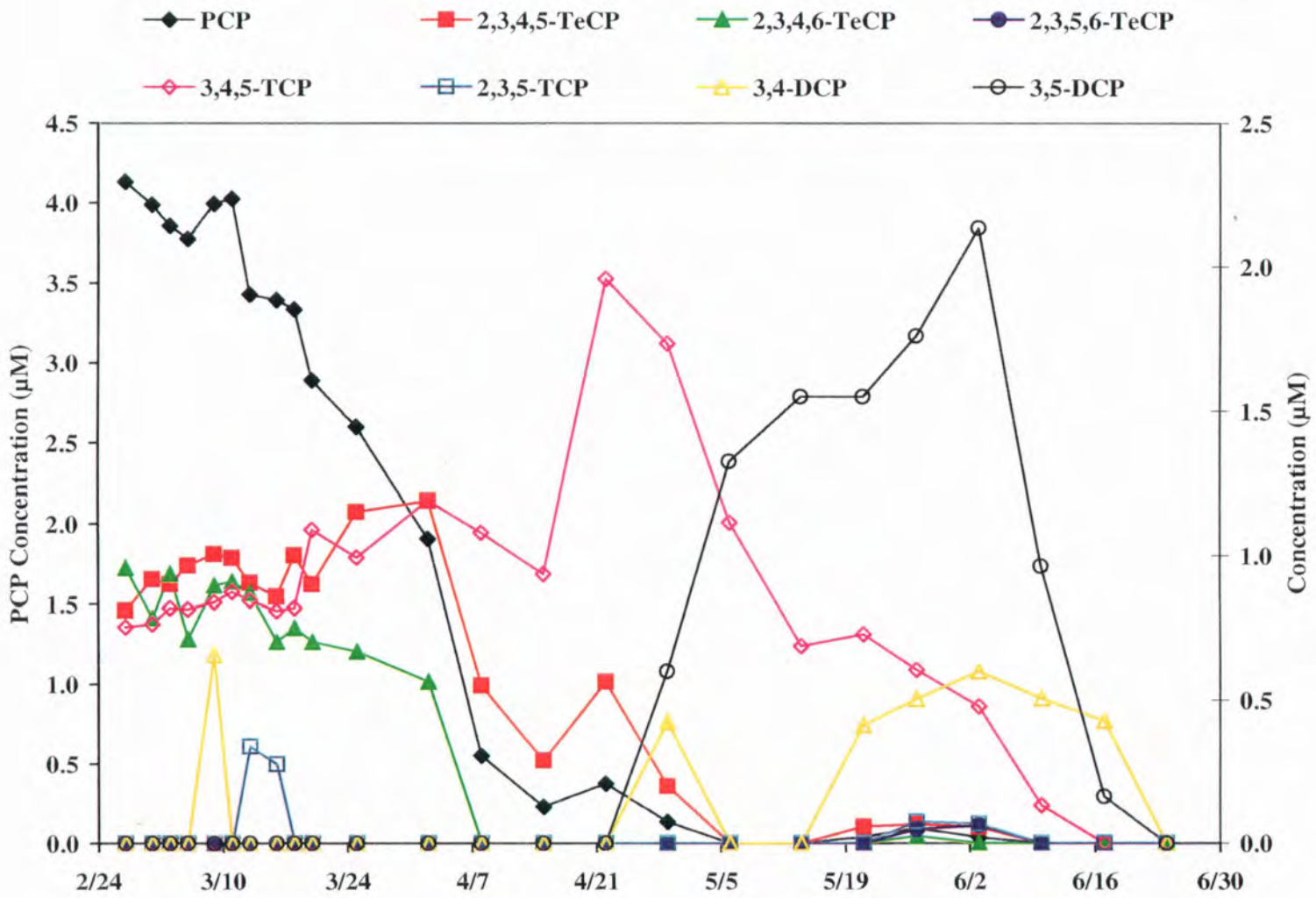

Figure 3.14 Chlorophenols observed during phase IV

measurement of 50 mV. Even in the presence of imitation vanilla flavoring provided at 100 mg COD/L, aerobic conditions prevailed in both treatment zones of the reactor assembly.

During phase III, an electrical failure of the nutrient injection and flow cell systems occurred. Nutrient injection and mixing was suspended for a period of two days before the system was reactivated. Over this period, no oxygen was added to the reactor system. When power was restored, apparent E_H measurements in the anaerobic zone showed a small deviation from observations made in the presence of nutrient addition and mixing. The deviation is shown graphically in Figure 3.9 and is located immediately prior to the change in experimental phases (III-IV). Albeit small, the decrease observed was concurrent in each treatment cell monitored. The magnitude of the changes in apparent E_H was more pronounced in measurements taken from the anaerobic treatment zone. In the absence of nutrient addition and mixing the flux of water into the zone A suggested a lower reduction potential naturally existed in the surrounding aquifer. Interestingly, in the absence of mixing, apparent E_H response supports the potential of a plug flow hydraulic regime and may further explain the greater magnitude of change observed in the anaerobic treatment zone. Following reactivation of the nutrient injection system, apparent E_H measurements in both treatment zones quickly returned to conditions observed prior to system upset. Reduced conditions required for PCP reductive dechlorination were not attained under the operational conditions evaluated.

To estimate the magnitude of PCP removal in phase III, the total molar mass of tetrachlorophenols observed in the system was normalized to the PCP concentration observed (Figure 3.14). An increase in the ratio at the onset of phase III suggests the transformation of PCP to tetrachlorophenols. However based upon the oxidative conditions observed in the reactor (Figure 3.9) the extent of PCP reductive dechlorination within the reactor is questionable. Nutrient injection to the treatment system operation could have easily impacted the conditions naturally present in the formation around the reactor well. Thus, it is more likely that PCP reductive dechlorination commenced in the surrounding aquifer material rather than within the oxidative environment in the reactor. With increasing radial distance from the oxidant supply anaerobic conditions, suitable for PCP may have developed in the surrounding formation. Transformation of PCP in the aquifer prior to the reactor inlet would have produced results consistent with those observed in phase III; a reduction in PCP loading rates to the reactor system with an associated increase in tetrachlorophenol concentrations. Further support for transformation outside of the reactor can be found in the lack of dechlorinated intermediate production in the system during phase III. Production of these intermediates was not observed until the oxidant supply was removed in phase IV (Figure 3.13) and apparent E_H measurements decreased to conditions favorable for reductive processes (Figure 3.9).

Rapid changes in the environmental conditions within the treatment zones illustrated the sensitivity of the reactor assembly to oxygen addition. Changes in the gas supply duration made during phase IV experiments were immediately evident in apparent E_H measurements in both treatment zones (Figure 3.9). The apparent E_H conditions in both treatment zones decreased at nearly identical rates. Concurrent with the

decrease in apparent E_H, PCP concentrations began to decrease while, concentrations of lower order chlorophenols increased (Figure 3.8 and Figure 3.13). PCP concentrations in the reactor continued to decrease with time. Complete biological removal of PCP occurred approximately three months after the reduction in oxygen addition. Degradation of PCP followed a seemingly straightforward pathway in the permeable barrier reactor.

For many reasons, it was not possible to quantitatively track all products of PCP reductive dechlorination. Therefore, construction of a standard stoichiometric mass balance around PCP was not performed. To obtain a better understanding of the observed transformations, the molar mass of each chlorophenol congener quantified was summed for each sampling interval. Individual species were then normalized to the total molar mass present at each sampling interval. Figure 3.15 presents chlorophenols observed in the reactor over the experimental phases as a function of their molar mass fraction. Evaluation of Figure 3.15 over the experimental phases supports the biological removal of PCP in the permeable barrier reactor. The relationship of decreasing PCP concentration and increasing metabolite concentrations is strong evidence to support biological PCP transformation in the permeable barrier reactor. Within phase III, removal of PCP is suggested in Figure 3.15. PCP degradation under aerobic conditions by a mixed culture has been reported by (Brown et al., 1986; Moos et al., 1983) and may explain the apparent concentration decrease. There are many metabolic products of aerobic PCP degradation. However, the first step of degradation is generally hydroxylation, which results in the formation of chlorocatechols. If chlorocatechol production had been significant, analytical methods used for chlorophenol measurements would have shown rouge peaks at unexpected retention times. Samples analyzed during phase III of the experiment provided no evidence to support the production of chlorocatechols through aerobic PCP degradation.

Metabolic products of PCP degradation in phase IV indicated that dechlorination through dichlorophenols occurred under the anaerobic conditions in the reactor. Although production of monochlorophenols was not observed, it is likely that production from dichlorophenols occurred. No accumulation of chlorinated phenolic products was observed which suggests complete reductive dechlorination of PCP in the reactor system under anaerobic conditions. Complete anaerobic PCP reductive dechlorination to phenol by a mixed culture has been reported by several groups (Bryant et al., 1991; Juteau et al., 1995a; Kennes et al., 1996; Mikesell and Boyd, 1986). Rapid *in-situ* transformation of PCP at the McFarland facility was achieved without the use of an aerobic treatment zone. Observation of PCP behavior and congener mass fraction over the five experimental phases clearly shows the impact of the process changes made during phase III and IV. Reduction of the oxidant supply seemed to be the key to initiate the rapid removal of PCP observed in phase IV. It is clear that when anaerobic conditions prevailed in the permeable barrier reactor, PCP degradation was efficient and complete.

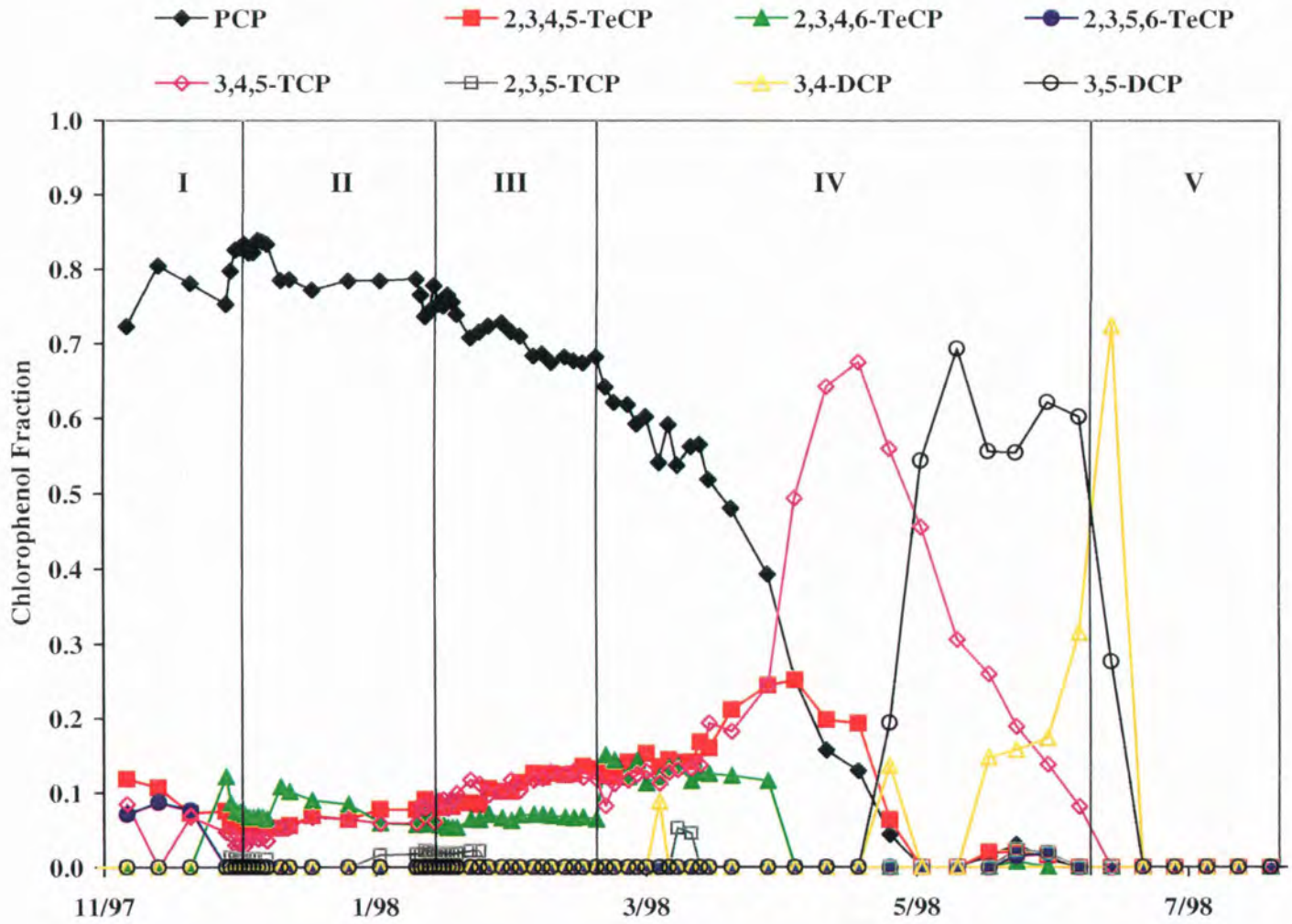

Figure 3.15 Chlorophenol mass fraction observed in an in-situ permeable barrier reactor

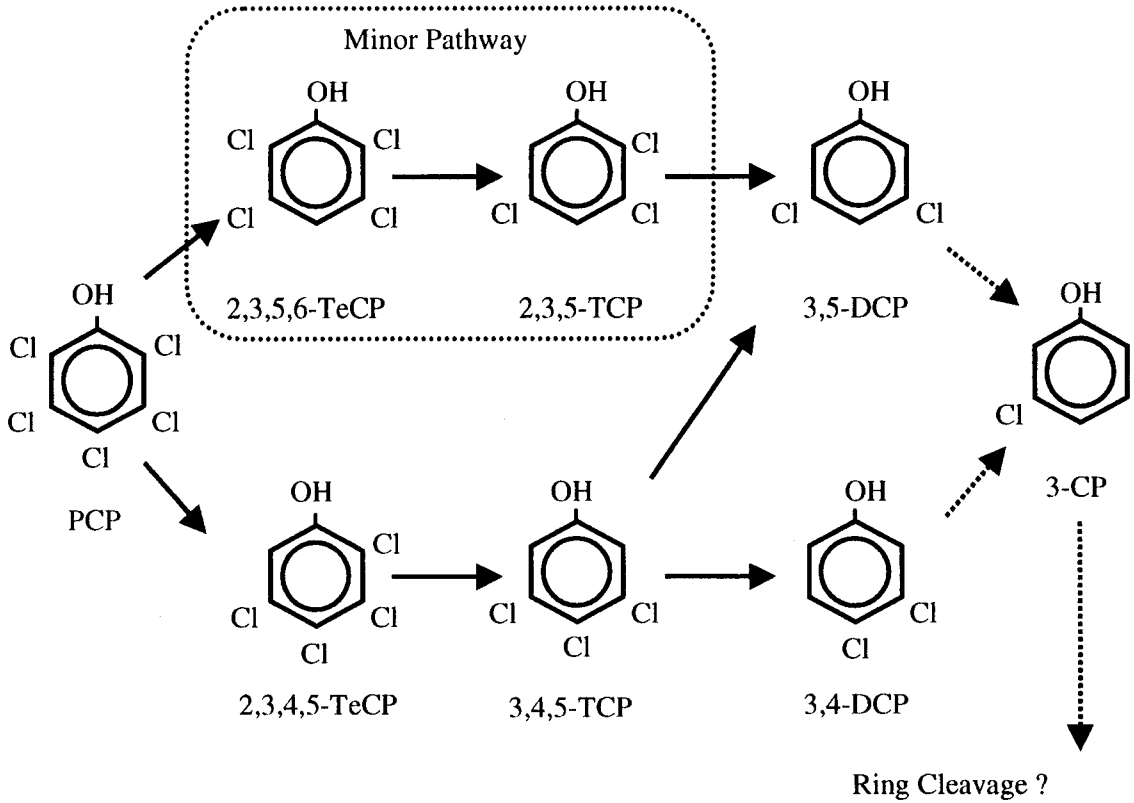

Figure 3.16 Reductive dechlorination pathway observed in an *in-situ* permeable barrier reactor

Pathway

Dechlorination of PCP followed the pathway shown in Figure 3.16. Primary degradation of PCP occurred by sequential *ortho* dechlorination to form 3,4,5-TCP. Degradation of 3,4,5-TCP yielded 3,4-DCP and 3,5-DCP which were further degraded in the system. While detection of monochlorophenols is possible with the analytical procedures used, special preparation steps were required for quantification. As such routine analysis for products of 3,4-DCP and 3,5-DCP degradation were not conducted. Evaluation of the metabolites identified indicates the ability of the population to perform dechlorinations at the *ortho*, *meta* and *para* positions. Degradation of 2,3,5,6-TeCP at the *ortho* position was the likely source of 2,3,5-TCP in the system. Dechlorination of 2,3,5-TCP may have resulted in the production of both 3,4-DCP and 3,5-DCP. Complete removal of 2,3,4,6-TeCP was observed; metabolic products of degradation were not evaluated. Production of 3,4-DCP from sequential *ortho* dechlorinations of 2,3,4,6-TeCP was hypothesized. The degradation pathway observed in the field was similar to observations made in serum bottle assays evaluated in the laboratory. Contradictory to laboratory observed pathways, initial dechlorination of PCP at the *para* position did not occur under field conditions. The presence of 2,3,5,6-TeCP as a technical impurity and the relatively low concentrations in the aquifer system prevented any inference of biological production from PCP dechlorination. Experiments conducted at the laboratory scale proved very useful in prediction of potential field biotransformations.

Controls

Parallel controls were not used during the pilot scale demonstration. With such great seasonal variations in contaminant loading, analysis and comparison of historical and experimental data were deemed the only acceptable indicators of biological PCP removal. Confirmation of biological transformation processes lies in the interpretation of the data collected during the phases outlined in Table 3.2. Analysis of site data collected prior to reactor installation provided no indication of natural PCP biotransformation. Chlorophenols observed in the aquifer system historically and during phases I and II indicated the presence of PCP and trace amounts of 2,3,4,5-TeCP, 2,3,5,6-TeCP and 2,3,4,6-tetrachlorophenol. The three tetrachlorophenols observed are likely impurities in the technical grade PCP rather than products of degradation. Qualitative analysis of LNAPL recovered from the aquifer revealed all three tetrachlorophenols historically observed in site groundwater were also present in the treating oil.

The absence of biotransformation during phase I, II and III (Figure 3.15) is supported by the stable concentration profiles of the tetrachlorophenols present in the reactor system. There was no evidence to support biological degradation of PCP under: natural aquifer conditions in phase I (Figure 3.10), in the presence of inoculum in phase II (Figure 3.11) or in phase III under an oxidizing environment with inoculum and electron donor (Figure 3.12). In phases I-III, tetrachlorophenols present never truly increased despite fluctuating PCP concentrations. Likewise, appropriate environmental conditions for PCP degradation (apparent E_H <-200 mV) was not achieved until the onset of phase IV of the demonstration

(Figure 3.9) when oxygen supply was decreased. Transformation of PCP in phase IV was a biologically mediated process and occurred by the process of reductive dechlorination. Degradation occurred in the presence of imitation vanilla flavoring and a reducing environment. Biotransformation is based on the chemical distribution of chlorophenols and environmental conditions observed in the reactor system. Chemical analysis of groundwater composition showed little change prior to phase IV. Removal of PCP in phase IV resulted in the production and removal of several dechlorinated intermediate compounds. Two of the compounds produced: 3,4-DCP and 3,5-DCP had never been observed at the site historically or during any previous phase of the pilot demonstration.

SUMMARY AND CONCLUSIONS

A bioremediation system for the *in-situ* degradation of pentachlorophenol was designed and constructed. The system was based upon a permeable barrier concept and used a large diameter well for the passive interception of PCP contaminated groundwater. Field demonstration of the treatment technology and subsequent biodegradation studies were conducted at the L.D. McFarland Facility in Eugene, Oregon. Biological treatment of PCP contaminated groundwater occurred in a cylindrical reactor that was installed within the casing of a large diameter well. The reactor assembly was installed at a depth corresponding to the screened interval of the well. Vertical partitions in the cylindrical unit created three zones for biological treatment process and three zones for nutrient addition and mixing. Biologically active zones were packed with municipal wastewater inoculated ceramic saddles. Imitation vanilla flavoring was supplied to the unit as an electron donor, mixing was accomplished through the addition of nitrogen and oxygen gas. The reactor was monitored with a custom designed pneumatic sampling system. Environmental conditions were measured and automatically logged in two positions in the treatment unit using recirculating flow cells.

Biodegradation of PCP was evaluated under several environmental conditions. There was no evidence to support natural PCP biodegradation. In the presence of the inoculated permeable barrier, PCP present in the groundwater was not degraded. Under oxidizing conditions in the presence of cells and imitation vanilla flavoring, PCP was not appreciable degraded in the permeable barrier reactor. PCP degradation was observed in the presence of imitation vanilla flavoring and cells. Environmental conditions measured in the treatment zones indicated that PCP biotransformation occurred under anaerobic conditions. Chemical speciation of PCP degradation products indicated reductive dechlorination was the primary mechanism of removal. Degradation of PCP *in-situ* was complete in the pilot scale demonstration at the L.D. McFarland facility. Results from the pilot demonstration study indicate that biological permeable barriers are an effective tool for the remediation of contaminated groundwater.

ACKNOWLEDGMENTS

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CHAPTER 4

FIELD AND LABORATORY COMPARISONS OF SUBSTRATE REQUIREMENTS FOR THE BIOREMEDIATION OF PENTACHLOROPHENOL-CONTAMINATED GROUND WATER

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ABSTRACT

The effect of supplemental electron donor concentration on the reductive dechlorination of pentachlorophenol (PCP) was evaluated in the field and in the laboratory. *In-situ* degradation studies were conducted in a PCP-contaminated aquifer using a biological permeable barrier reactor. Imitation vanilla flavoring served as the electron donor for this study. It was supplied to the reactor system to provide a supplemental carbonaceous oxygen demand (COD) of 0, 10, 50 and 100 mg/L. Changes in reactor operation and performance were evaluated as a function of supplemental COD. Laboratory comparisons of PCP degradation were made using batch serum bottles incubated at 14°C and amended with supplemental COD of 0, 10, 25, 50, 100 mg /L. Serum bottle assays were conducted in duplicate and parallel poisoned controls were constructed to assess abiotic PCP degradation. Results from the field and laboratory studies were complementary. In the presence of an exogenous electron donor, PCP degradation was independent of donor concentrations supplied. In the laboratory studies, the rate of PCP removal was independent of supplemental donor concentrations of 10 mg COD/L or above. However, PCP degradation at comparatively slower rates was observed in the absence of electron donor addition under both field and laboratory conditions. Under laboratory conditions, PCP was degraded to 3,4,5-trichlorophenol (3,4,5-TCP) by the sequential *ortho* reductions. Concentrations of 3,4,5-TCP accumulated in the serum bottles at COD treatments of 0, 10, 25 and 50 mg/L. Small amounts of 3,4,5-TCP were transformed to 3,5-dichlorophenol in serum bottles evaluated with supplemental COD at 100 mg/L. Conversely, PCP degradation in the permeable barrier reactor was complete; no accumulation of intermediate products was observed. Results indicate that a 10-fold decrease in the supply rate of supplemental electron donor has no appreciable effect on the efficiency of *in-situ* PCP degradation in a biological permeable barrier. Findings of the study illustrate the importance of parallel laboratory and field based studies for the cost-effective operation of *in-situ* biological treatment systems.

RESEARCH OBJECTIVES

In studies of anaerobic pentachlorophenol (PCP) reductive dechlorination, toxicity of the target compound, PCP is often of more concern than the concentration of supplemental electron donor. Numerous biodegradation studies have focussed on the efficiency of PCP degradation as a function of the contaminant loading rate (Godsy et al., 1986; Jin and Bhattacharya, 1996; Juteau et al., 1995a; Larsen et al., 1991; Mohn and Kennedy, 1992). While PCP toxicity to the anaerobic culture is of great importance to the success of the biological remediation strategy, the contribution and potential effects of supplemental electron donor addition cannot be casually overlooked. Few studies have focussed on the effects of electron donor concentration on PCP reductive dechlorination. To better understand the relationship between electron donor concentration and reductive dechlorination, PCP degradation was evaluated as a

function of supplemental electron donor concentration. Parallel degradation studies were conducted in the field and laboratory. Field based experiments were conducted in a pilot scale biological permeable barrier reactor. The reactor was fabricated to fit within the casing of a large diameter well that was constructed in a PCP-contaminated aquifer. Laboratory studies were conducted in batch serum bottles. Specifically, this comparison study was undertaken with the following objectives:

- Determine the effect of electron donor concentration on the reductive dechlorination of PCP under field and laboratory conditions.
- Optimize operation of the pilot scale reactor through the identification of threshold electron donor concentrations needed to support PCP reductive dechlorination.
- Investigate the potential for the use of alternate electron donors in the pilot scale permeable barrier reactor.
- Evaluate the pathway of PCP reductive dechlorination under field and laboratory conditions.

INTRODUCTION

Environmental contamination from chlorinated phenolic compounds poses serious threat to groundwater quality in many areas of the United States. Desired for its biocidal properties, pentachlorophenol (PCP) has been primarily used for the chemical preservation of wood products. While minor in comparison to wood preservation, PCP has also been used for a variety of industrial and agricultural purposes (Crosby et al., 1981; Guthrie et al., 1984). In addition to industrial and agricultural usage, PCP may be released to the atmosphere by combustion processes or to the aquatic environment through chlorine bleaching of wood pulp (Häggblom, 1990). The widespread environmental distribution of PCP, high toxicity characteristics and low Maximum Contaminant Level (MCL=1μg/L; (Keith and Telliard, 1979)) provides ample cause for the development of remediation technologies capable of aquifer restoration.

Microbial degradation of chlorophenols, including PCP, has been studied using pure cultures and microbial consortiums under a wide array of environmental conditions. Degradation mechanisms of PCP and associated chlorinated phenolic compounds are well understood and have been evaluated under both aerobic and anaerobic conditions (Häggblom, 1990; Häggblom, 1992; Mohn and Tiedje, 1992). Under anaerobic conditions, reductive dechlorination of PCP is favored in the presence of a suitable electron donor. The reductive dechlorination of PCP has been observed in the absence of an extrinsic electron donor (Boyd and Shelton, 1984; Boyd et al., 1983; Fathepure et al., 1988; Mikesell and Boyd, 1985; Mikesell and Boyd, 1986; Mikesell and Boyd, 1988). However, the addition of an external electron donor has been shown to enhance PCP reductive dechlorination by anaerobic consortiums. Effective PCP reductive dechlorination in the presence of an exogenous electron donor has been shown with hydrogen (Madsen and Aamand, 1991), acetate (Nicholson et al., 1992; Woods et al., 1989) and propionate (Jin and

Bhattacharya, 1996). PCP reductive dechlorination with electron donors requiring anaerobic fermentation has also been shown with phenol (Duff et al., 1995) and with mixtures of phenol and glucose (Hendriksen et al., 1992) or phenol an ethanol (Larsen et al., 1991). While the benefits of supplemental electron donor addition have been realized, the minimum threshold concentration in which donor addition contributes to PCP reductive dechlorination remains unclear.

In studies of anaerobic PCP reductive dechlorination, toxicity of the target compound, PCP is often of more concern than that of the supplemental electron donor concentration. Numerous biodegradation studies have focussed on the efficiency of PCP degradation as a function of the contaminant loading rate (Godsy et al., 1986; Jin and Bhattacharya, 1996; Juteau et al., 1995a; Larsen et al., 1991; Mohn and Kennedy, 1992). While PCP toxicity to the anaerobic culture is of great importance to the success of the biological remediation strategy, the contribution and potential effects of supplemental electron donor addition cannot be casually overlooked. Few studies have focussed on the effects of electron donor concentration and PCP reductive dechlorination. Duff et al., 1995 individually evaluated the toxicity of PCP and phenol using acetoclastic toxicity assays. Phenol concentrations less than 500 mg/L showed no signs of toxicity to the anaerobic cultures used in the reactor system. The tests were conducted in an attempt to characterize consortium toxicity to PCP and phenol, for reductive dechlorination studies conducted in an upflow anaerobic sludge blanket (UASB) reactor. A USAB fed a mixture of electron donors at influent carbonaceous oxygen demand (COD) values from 10-1.2 g/L showed that a decrease in influent COD did not change PCP removal efficiency (Wu et al., 1993). A reduction in the growth rate of the anaerobic granules was the only consequence noticed by the reduction in USAB influent COD. Stable degradation of PCP was observed under varying influent COD in a fluidized-bed granular activated carbon rector supplied ethanol as electron donor (Khodadoust et al., 1997). In all of the reactor systems studied however, no effort was made to determine the minimum influent COD capable of supporting PCP reductive dechlorination.

Coincident to a minimum beneficial supplemental electron donor concentration, there also exists a point were repeated or continued donor addition becomes inhibitory to the degradation process. When increasing phenol concentrations were supplied to a 4-chlorophenol degrading anaerobic consortium, inhibition of the reductive dechlorination process occurred. Degradation of 4-chlorophenol was significantly attenuated and commenced only after phenol was removed. High phenol concentrations completely inhibited the reductive dechlorination of 4-chlorophenol (Zhang and Wiegel, 1990). Excess nutrients were considered inhibitory to a PCP degradation by a methanogenic consortium evaluated in a continuous stir tank reactor (Chang et al., 1998a). In addition to toxicity issues associated with supplemental electron donors, thermodynamics may affect the overall efficiency of the biotransformation processes. Studies using an acetogenic benzoate degrading culture showed the continued addition of acetate inhibitory to the degradation process. The lack of an appropriate acetate sink thermodynamically limited the rate of benzoate degradation (Dolfing and Tiedje, 1988). Whether a function of the cultures

nutritional requirements, toxicity or thermodynamic properties, there is clearly an optimal range for the addition of a supplemental electron donor to a PCP degrading consortium.

Aside from the considerations of the physiological factors associated with the use of supplemental electron donors is their potential cost and method of field application. For an *in-situ* bioremediation system, effective control of operational costs is directly linked to the supply of electron donor. From the operation perspective is the purchase and consumption rate of the raw chemical feedstock supplied to the system while, maintenance considerations center around the potential for biological fouling of supply equipment, injection wells and ultimately the aquifer structure. Pulsed substrate addition at the field scale has helped limit biological fouling processes (Hooker et al., 1998; Peyton, 1996; Hopkins et al., 1993). However, routine maintenance and cleaning procedures are still required to keep an *in-situ* treatment system operating at design capacity (McCarty et al., 1998). Operation and maintenance costs of the *in-situ* treatment system again illustrate the need for optimization studies relative to supplemental electron donor addition and PCP reductive dechlorination.

The laboratory setting is the ideal platform in which nutritional the requirements of PCP degrading culture can be evaluated and optimized. The reliability of process and system control found within the laboratory offer the ability to scientifically a range suitable substrate concentrations that consider the nutritional and economic requirements of a field based treatment system. Hopkins et al., 1993 illustrated the power of companion laboratory and field studies with the *in-situ* degradation studies of trichloroethylene (TCE) conducted at Moffett Naval Air Station, Mountain View, CA. Findings from the laboratory were used to investigate the cometabolic removal of TCE, phenol and oxygen *in-situ*. Laboratory tests showed a phenol oxidizing bacteria possess a much higher transformation capacity of TCE than the methane oxidizers. Subsequent field studies verified this laboratory-based prediction. Laboratory studies further identified a relationship between phenol and TCE whereby, increasing TCE concentrations could be effectively degraded by increasing phenol supplied to the system. Again, laboratory predictions were accurate in describing the behavior of TCE degradation at the field trial. Increased phenol concentrations provided greater TCE removal efficiencies. In an effort to design and operate economical *in-situ* biological treatment systems, few information sources can compare with companion laboratory and field studies.

Incorporating anaerobic degradation principles, an *in-situ* remediation technology for PCP-contaminated groundwater has been developed, designed and constructed at the pilot scale. The treatment strategy is based on the concept of a permeable biological barrier and is housed within a large diameter well installed in a PCP-contaminated aquifer in Eugene, Oregon. Details of the reactor construction, operation and site characterization have been provided elsewhere (Cole and Woods, 2000b). Degradation of PCP by an anaerobic consortium is supported through the addition of a supplemental electron donor. In an effort to characterize the nutritional requirements, controlled laboratory serum bottle assays were

conducted over a range of substrate concentrations investigated in a pilot scale permeable barrier at the L.D. McFarland facility. Specifically, this study was undertaken to determine the effect of electron donor concentration on the *in-situ* reductive dechlorination of PCP; to determine the optimum electron donor concentration for barrier operation and compare field and laboratory reductive dechlorination pathways.

MATERIALS AND METHODS

Anaerobic pentachlorophenol degradation as a function of electron donor supply was evaluated under laboratory and field conditions. Field based experiments were conducted at the pilot scale using a custom designed permeable barrier reactor. The reactor assembly was fabricated to fit within the casing of a large diameter well that was constructed in a PCP-contaminated aquifer at the L.D. McFarland facility in Eugene, Oregon. Details of the reactor and the supporting control systems are outlined in Chapter 3. PCP concentrations in the reactor were evaluated using four concentrations of the system electron donor, imitation vanilla flavoring. Laboratory studies were conducted in batch serum bottles. Five concentrations of electron donor were evaluated and bracketed concentration ranges investigated in the field. Tests were conducted in duplicate for each concentration evaluated. Parallel controls were used to discern abiotic and biological PCP removal. Progress curves constructed over the experiment duration were used to monitor chlorophenol degradation rates and pathways. Selected components of the imitation vanilla were also monitored to ensure the systems were not electron donor limited. Finally, gas production in the active and control bottles was measured on a volumetric basis.

Inoculum

Consortia used in the serum bottle assay were harvested from the pilot scale permeable barrier reactor system installed at the L.D. McFarland facility in Eugene, Oregon. The mixed anaerobic culture originated as a combination of return secondary sludge from an activated sludge system and supernatant from the anaerobic sludge digester. Culture development and adaptation within the reactor system to the physical and geochemical conditions produced a robust consortium capable of rapid anaerobic PCP biotransformation. Groundwater was pumped from a central location in the reactor and dispensed to a 4-liter flask that was continuously purged with nitrogen gas. Natural aquifer temperature varies seasonally from 12°C to 16°C. Groundwater used for the serum bottle assay measured 14°C during collection. The flask was sealed and chilled on ice for transport to the laboratory. Upon arrival, the flask containing cells was transferred to an anaerobic glove box. The cell suspension was then homogenized by vigorous mixing. An aliquot of the cell mixture, used for parallel sterile controls was removed from the glove box for autoclave sterilization.

Laboratory Experimental System

Glass 300 ml serum bottles were used to conduct the degradation study. The bottles were cleaned in a 50% v/v sulfuric acid solution, triple rinsed with de-ionized water and autoclaved. Each bottle contained 50 ml of headspace and 250 ml of liquid. Bottles were screw capped with Teflon® faced butyl rubber stoppers. The serum bottle sets were constructed at room temperature in the confines of an anaerobic glove box. Bottles sets were constructed at carbonaceous oxygen demand COD values of 0, 10, 25, 50 and 100 mg/L. The supplemental COD was supplied in the form of imitation vanilla flavoring. To match the aquifer conditions at the field site, an aqueous PCP solution was added to the serum bottle sets to provide an initial concentration of 4.8μM.

Field Experimental System

A detailed description of operation and control of the mixing and nutrient supply system in the permeable barrier reactor is found in Chapter 3. In field based experiments, electron donor was continuously pumped to the reactor mixing zones through 1/8" O.D. (3.2 mm) Teflon® tubing and two FMI QG-6 positive displacement pumps, Fluid Metering Inc. (Oyster Bay, NY). Two standard size gas cylinders and two-stage regulators were used to supply low pressure mixing gas to the diffuser assemblies. Mass flow of the mixing gases was controlled by two adjustable electric solenoid valves Cole-Parmer® (Vernon Hills, IL). Concentration of the electron donor in the reactor was controlled by changing the COD of the imitation vanilla stock injected. Under field conditions, four electron donor concentrations (100, 50, 10, and 0 mg COD/L) were evaluated.

Serum bottle preparation for each COD treatment was identical: 235 ml site groundwater, 15 ml PCP stock and an appropriate volume of imitation vanilla flavoring were added to the sterilized bottles. Controls were constructed in open air: 235 ml sterilized groundwater, 15 ml PCP stock and imitation vanilla flavoring to provide 50 mg COD/L were added to the sterilized bottles. In addition to sterilization, controls were also chemically poisoned with mercuric chloride. The bottles were capped, shaken and immediately sampled for initial PCP concentration. The serum bottles were removed from the glove box and purged with nitrogen to flush the headspace of hydrogen captured during bottle closure in the anaerobic chamber. Purge gas was stripped of residual oxygen by in line contact with copper filings at 450°C. The serum bottles were then placed in an incubator at 14±2°C.

Laboratory Sampling Procedure

The serum bottles and experimental controls were sampled at room temperature with time to monitor chlorophenol degradation. Gas production was measured with a 5 ml luer tip syringe (Popper & Sons, New Hyde Park, NY). The syringe walls were first lubricated with de-ionized water to allow easy plunger movement. Air present in the syringe barrel was expelled and a new syringe needle was attached. The serum bottle septa were then punctured with the syringe. Displacement of the plunger indicated gas

production since the last sampling interval. Liquid samples were collected from the bottles with 100 µl syringes (Hamilton Co., Reno, NV) and were immediately prepared for chlorophenol analysis.

Field Sampling Procedure

Using the pneumatic sampling principle previously outlined in Chapter 4 aqueous samples were collected from the permeable barrier reactor with time. Samples were collected from all locations in the reactor and dispensed into 4 ml amber vials with Teflon® faced screw caps. Samples were stored on ice for transport to the analytical laboratory. Field samples were analyzed for chlorophenol concentration upon laboratory arrival.

Chemical Sources

Pentachlorophenol (purity > 99.9%) was obtained from Sigma Chemical Co. (St. Louis, MO) and was used without further purification. Individual components of the imitation vanilla flavoring were obtained from Aldrich Chemical Co. (Milwaukee, WI). All were reagent grade and possessed purity greater than 99%. Other chemicals consumed over the course of the experiment were obtained from Mallinckrodt Co., (Paris, KY) or EM Science, (Cherry Hill, NJ). Chlorophenol analytical standards were obtained from Ultra Scientific Inc., (North Kingston, RI).

Analytical Procedures

Chlorophenol samples were acetylated and extracted into hexane using a modification of the method developed by (Voss et al., 1980) and the National Council of the Paper Industry for Air and Stream Improvement (1981). Extractions were conducted as follows: 500 µl of a solution containing 30.4 g/L K_2CO_3 and 250 µg/L 2,4,6 tribromophenol (an internal standard) was combined with a 100 µl sample from the serum bottles in a disposable glass culture tube with a Teflon® faced cap. 100 µl of acetic anhydride was added and the tube was mechanically shaken for 20 minutes. 1 ml of chromatographic grade hexane was added and the tube was shaken for an additional 20 minutes. Hexane was removed from the tube and transferred to a 2 ml amber glass vial. The vial was sealed with a Viton® faced crimp cap. Vials were immediately loaded for analysis by capillary gas chromatography.

Chlorophenols were quantified on a Hewlett Packard 5890A gas chromatograph. Automated 1 µl injections were made on the inlet, which was operated, in a splitless configuration. A Hewlett Packard 3392A integrator handled acquisition and signal processing from the 63Ni Electron Capture Detector (ECD). Separation of chlorophenol congeners was accomplished on a DB-5 fused silica capillary column (30m x 320µm I.D. x 0.25µm film; J & W Scientific, Folsom, CA). Helium provided at 35 cm/s served as the column carrier gas. A 95/5 blend of argon/methane at 75 ml/min was used for detector make-up. The instrument was operated as follows: initial temperature of 45°C was held for 2 minutes; the temperature was then increased 25°C/min to 140°C and held for 5 minutes; the oven was then increased 5°C/min to

245°C where it was held for 10 minutes. Solids concentrations in the batch cell cultures were analyzed for total and suspended solids using standard methods 2540D and 2540E (Association, 1989).

RESULTS: LABORATORY

The effect of COD on the reductive dechlorination of PCP was evaluated in serum bottles under laboratory conditions. COD in excess of background was supplied by imitation vanilla flavoring at concentrations of 10, 25, 50, and 100 mg/L. Through the absence of supplemental electron donor addition, PCP reductive dechlorination was also evaluated under natural background COD concentrations which around 14 mg/L in the reactor location. Sterile controls were constructed with 50 mg COD/L to evaluate the potential of abiotic PCP degradation. The parallel control and each COD treatment were evaluated in duplicate. To facilitate data presentation, average values among COD treatments are shown. In all figures shown, chlorophenol concentration in micromolar (μM/L) is presented as a function of time in hours.

Pentachlorophenol Degradation

A comparison of average PCP removal among duplicates in the various COD treatments and parallel controls is presented in Figure 4.1 There was slight variability among initial PCP concentrations in the treatments evaluated. Initial PCP values ranged from 4.04 μM to 3.58 μM. Among the COD treatments evaluated, there was no clear difference in PCP removal during the first 16 hours. PCP removal from the initial conditions to hour 16 measured 18% in the controls and averaged 25±6% in biologically active systems. At 39 hours, subtle deviations in treatment performance are visible. PCP concentrations in the experimental control stabilized while degradation in the remaining treatments continued. A pronounced shift in PCP removal in the background COD treatment relative to the other supplemental COD treatments also became evident. With increasing time, no appreciable removal of PCP in the parallel control was observed. From 39 to 519 hours, PCP concentrations in the control treatment averaged 3.08±0.08 μM. Performance of the individual COD treatments evaluated was nearly identical after 39 hours with the exception of serum bottles lacking supplemental COD. PCP degradation was observed in the treatment containing only natural background COD. However, the rate of transformation was slower than systems supplemented with exogenous electron donor. The trends in PCP degradation as a function of COD treatment are clear in Figure 4.2. Upon experiment termination at 519 hours, only the treatment with 100 mg/L supplemental COD removed 100% of the initial PCP. Transformation of PCP in bottles with 50, 25, and 10 mg/L supplemental COD was not significantly different and measured 99.8%, 99.8% and 99.2%, respectively. Although incomplete PCP transformation was observed in the serum bottles evaluated with natural background COD, nearly 85% of initial PCP mass was removed.

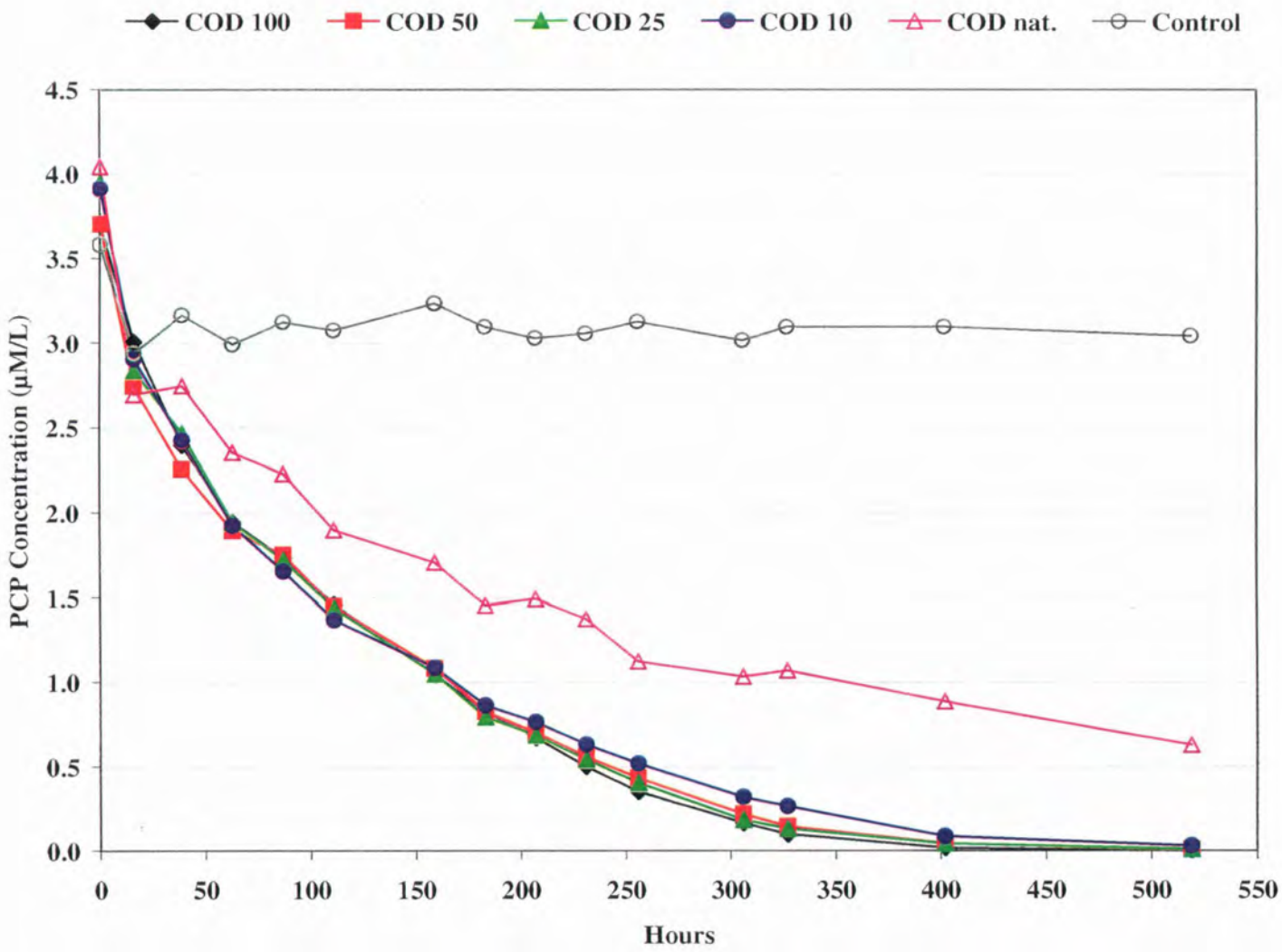

Figure 4.1 Average PCP concentration as a function of supplemental COD

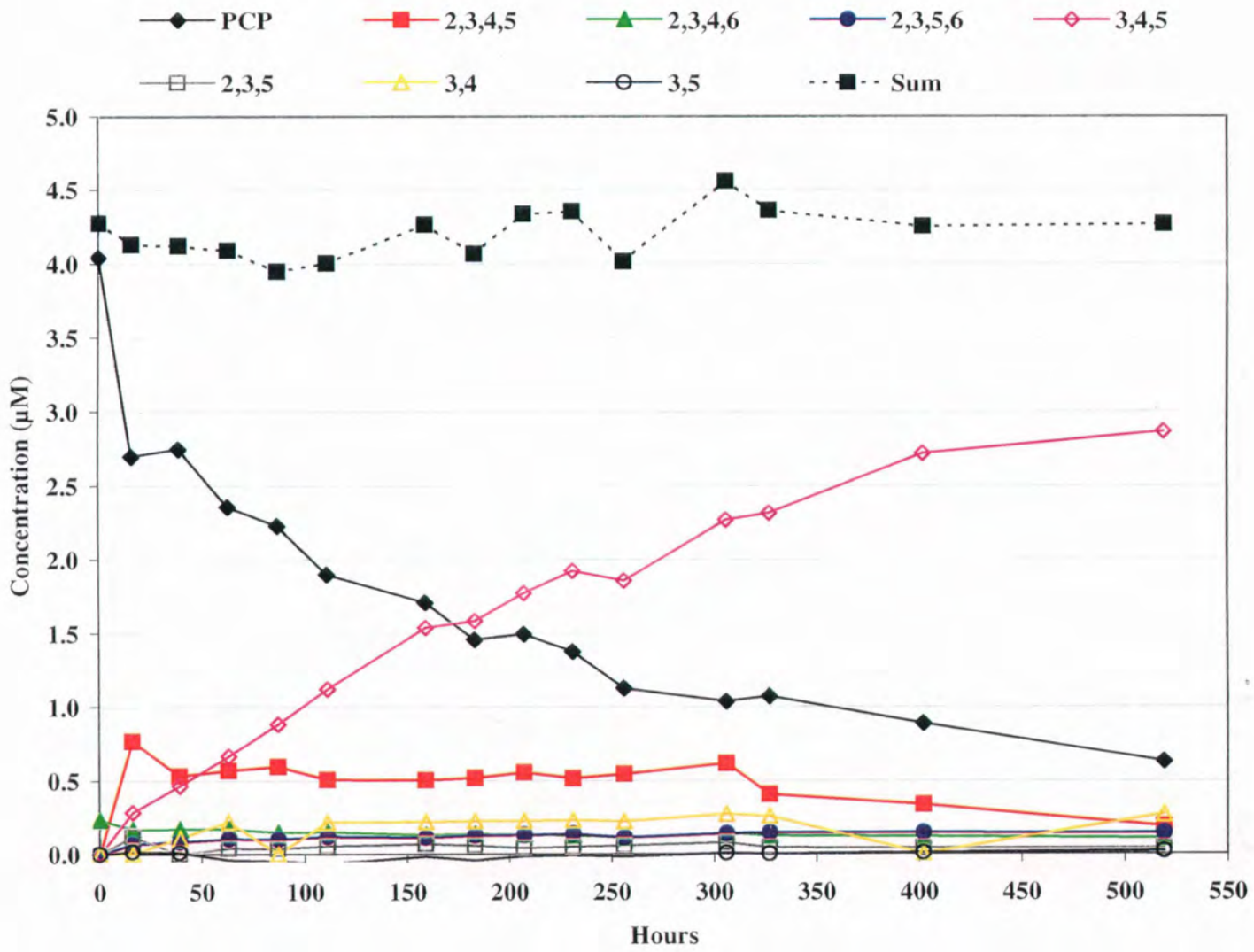

Figure 4.2 Reductive dechlorination pathway observed with background COD

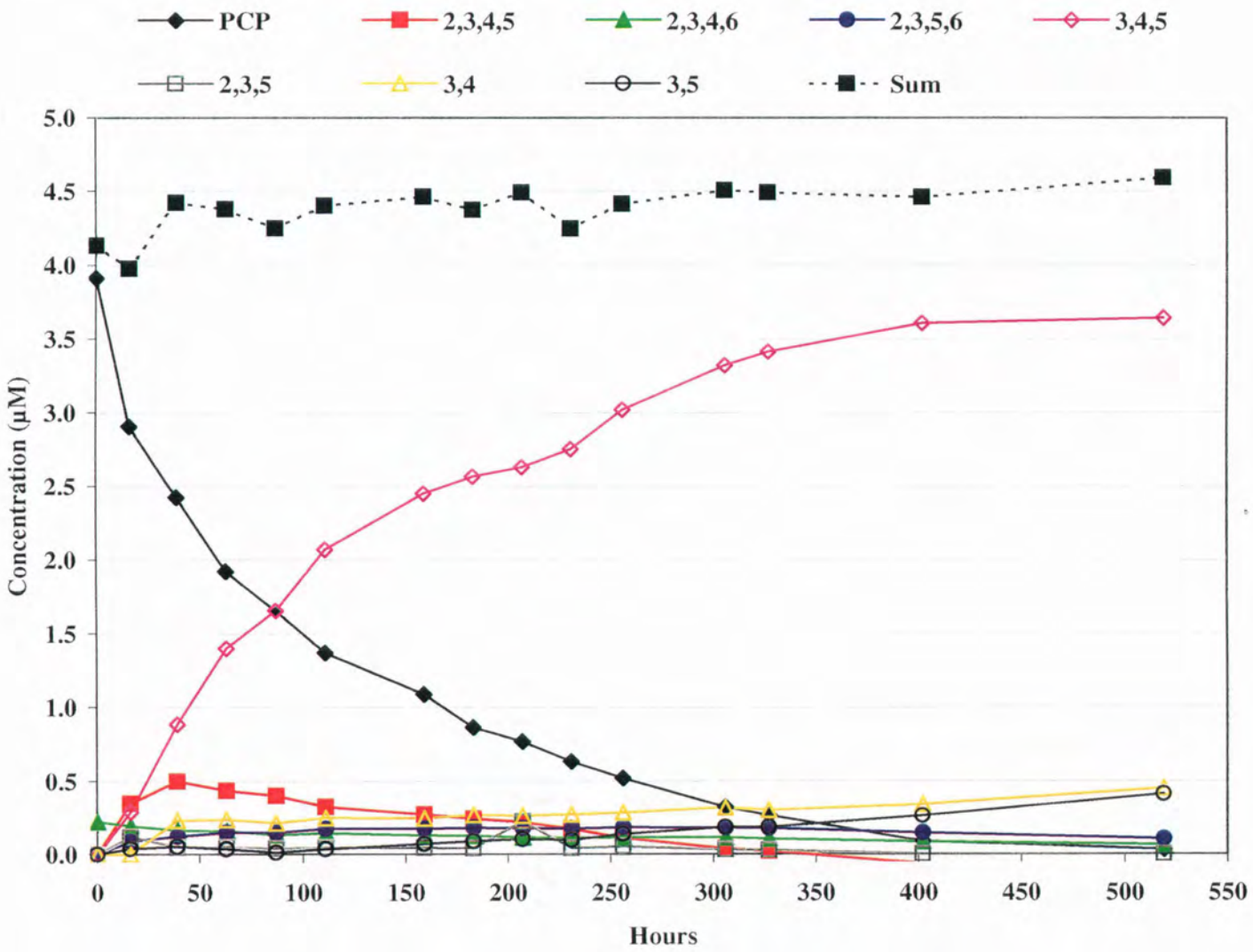

Figure 4.3 Reductive dechlorination pathway observed with 10 mg/L supplemental COD

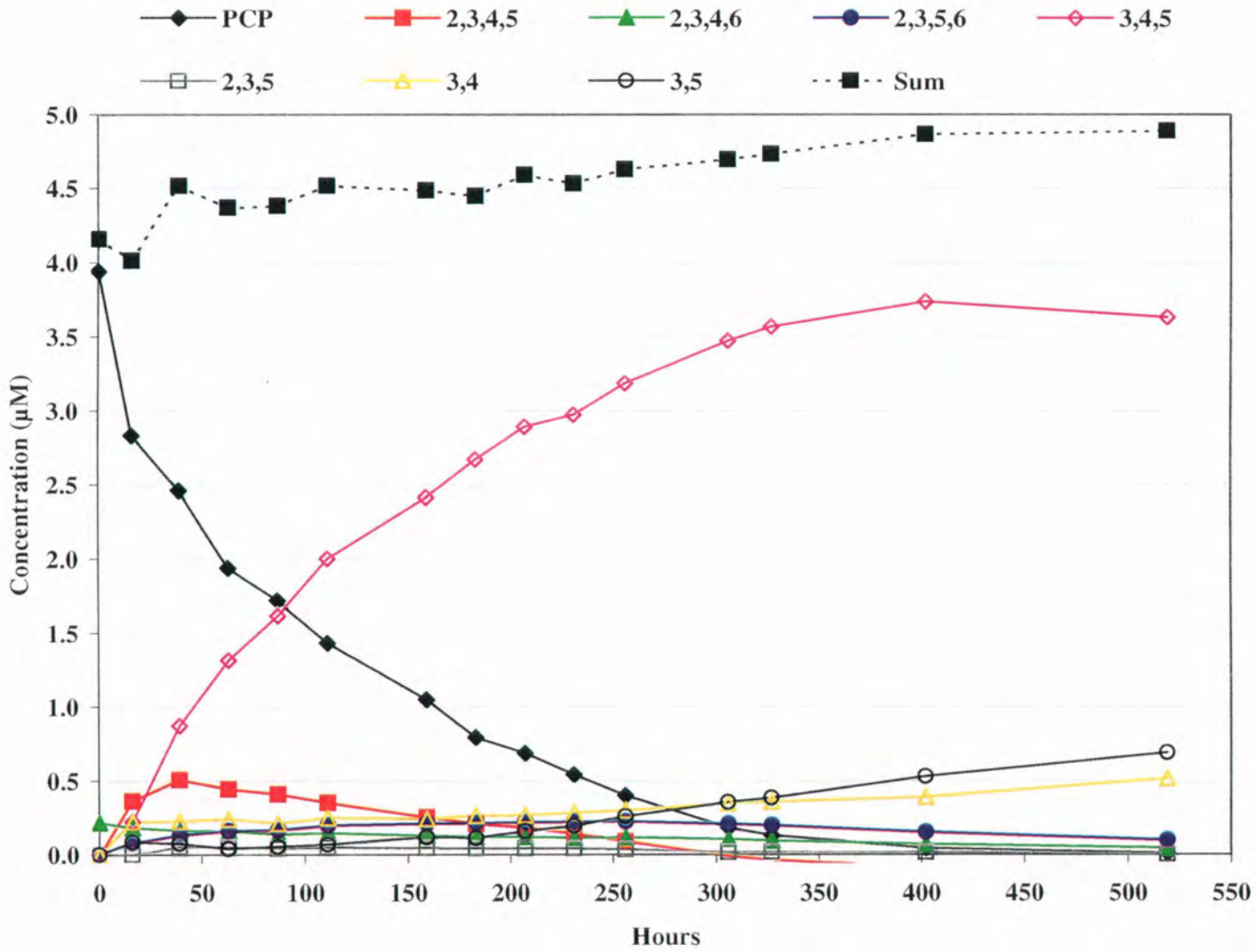

Figure 4.4 Reductive dechlorination pathway observed with 25 mg/L supplemental COD

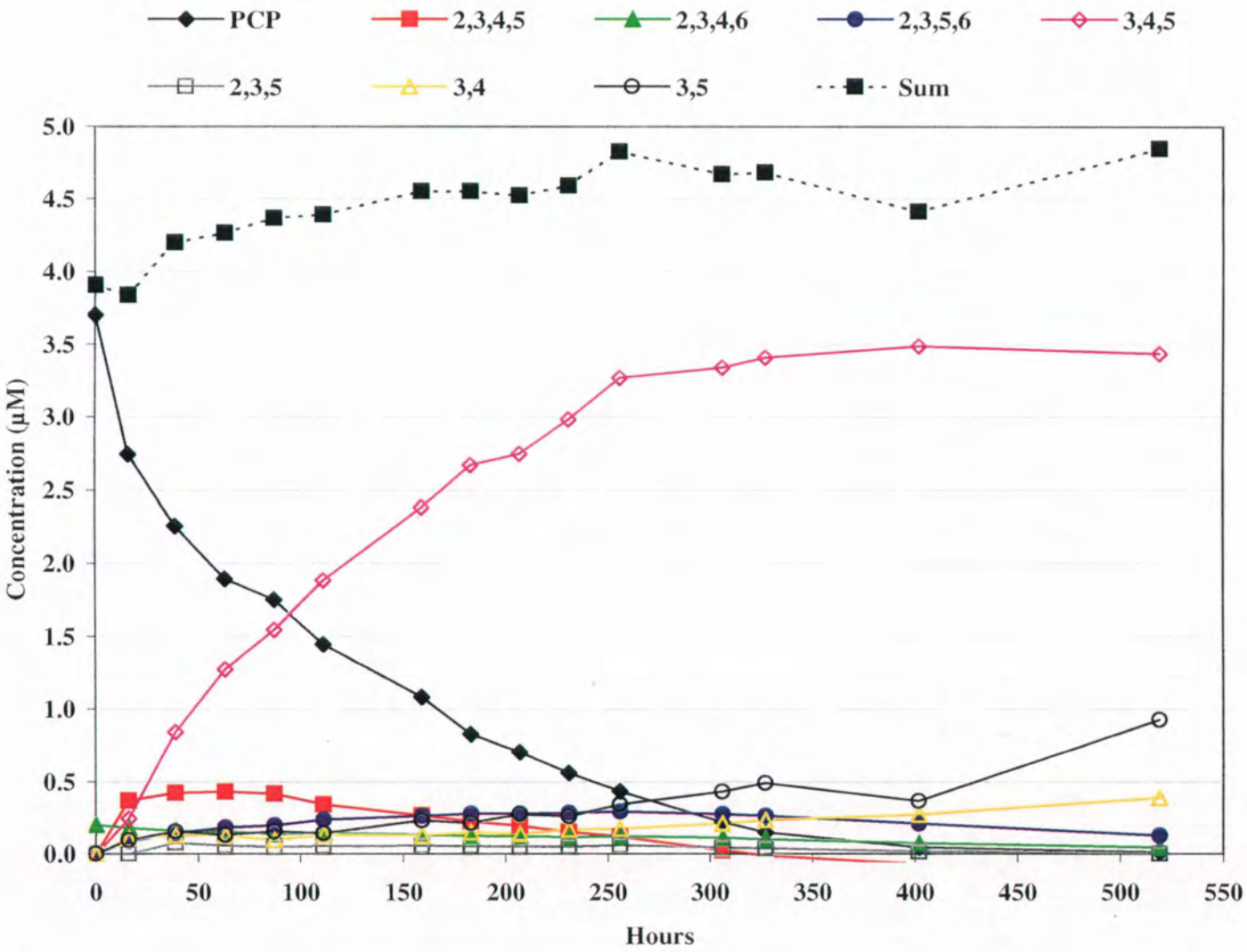

Figure 4.5 Reductive dechlorination pathway observed with 50 mg/L supplemental COD

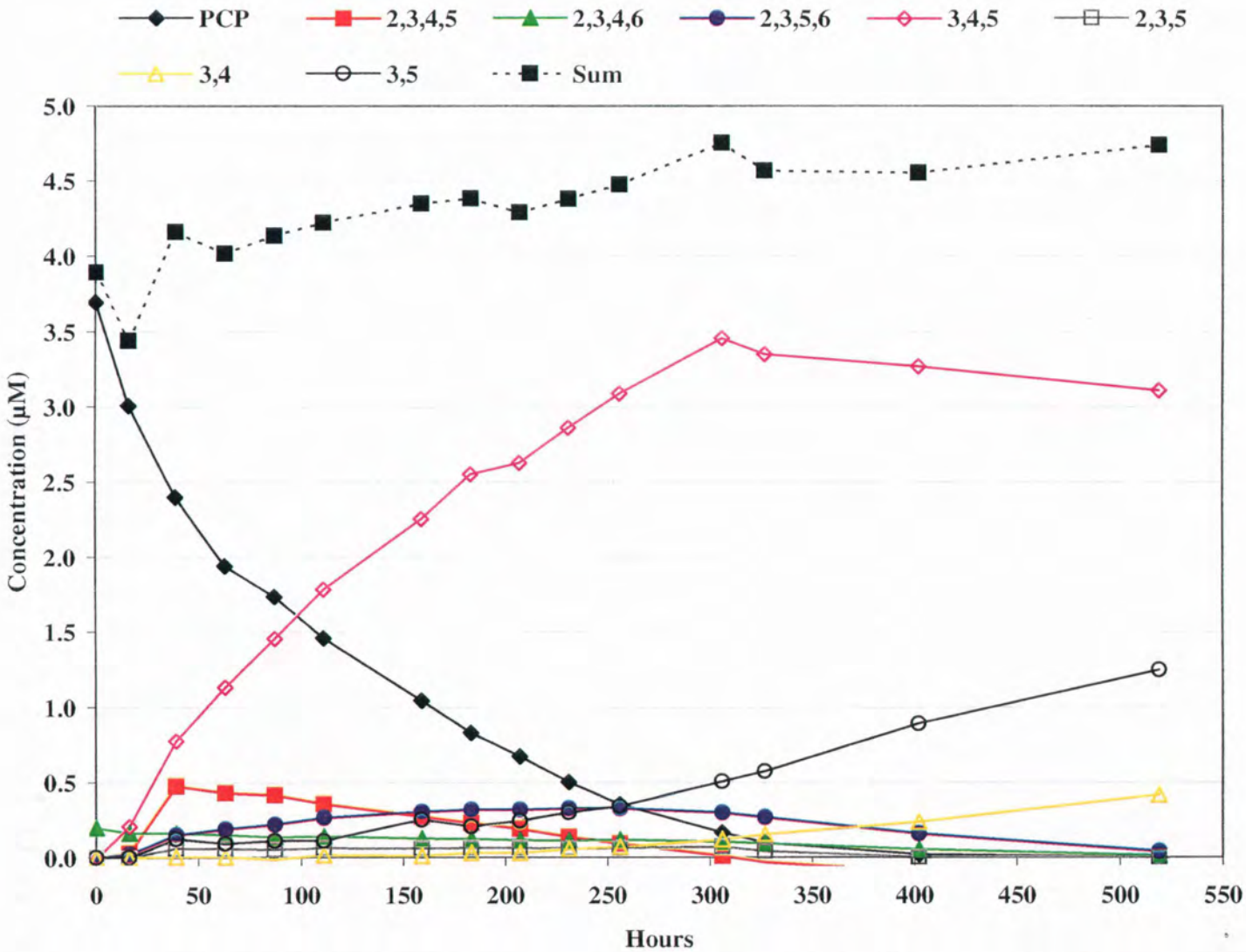

Figure 4.6 Reductive dechlorination pathway observed with 100 mg/L supplemental COD

Observed Transformation Pathway

The extent of chlorophenol removal was nearly identical in all COD treatments evaluated. Under natural background, 10, 25, 50 and 100 mg/L supplemental COD treatments, chlorophenols observed with time are shown in Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6, respectively. The pathway of PCP degradation in all COD treatments was identical. Initial degradation of PCP yielded 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), an *ortho* dechlorination product. Production of the *para* dechlorination species 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) was also observed at greatly reduced concentrations. Degradation of 2,3,4,5-TeCP was immediate and evidenced through the formation of 3,4,5-trichlorophenol (3,4,5-TCP), a product of sequential *ortho* PCP reductive dechlorination. In all treatments investigated, 3,4,5-TCP was the predominant metabolic product of PCP reductive dechlorination.

In Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6 mass balance construction over the chlorophenols observed adequately accounts for the total mass of PCP initially present in the system. Table 4.1 provides a comparison of the average chlorophenol mass to the initial PCP present in the COD treatments evaluated. The best correlation, 3.9% was observed with the system containing only background COD. Mass balance closure in treatments containing 50 and 100 mg COD/L was similar and measured 16.6% and 13.9 %, respectively. With the exception of the background COD treatment, production of 3,4,5-TCP was measured in near stoichiometric amounts to the initial mass of PCP present in the serum bottles. Ratios of maximum observed 3,4,5-TCP concentrations to that of initial PCP concentrations for COD treatments of 10, 25,50 and 100 mg/L yield 0.931, 0.949, 0.942 and 0.937, respectively. In all treatments, which contained supplemental COD, measured concentration ratios closely support a theoretical stoichiometric value of 1.00 indicating PCP reductive dechlorination proceeds almost exclusively through sequential *ortho* dechlorinations. Transformation of PCP to 3,4,5-TCP in the background COD system lacked the efficiency of electron donor amended systems. Upon experiment termination, the ratio of maximum 3,4,5-TCP to initial PCP concentration measured was 0.709.

In Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6 accumulation of 3,4,5-TCP is apparent. Figure 4.6 suggests the removal of 3,4,5-TCP from a maximum observed concentration of 3.82 μM at 306 hours. Removal of 3,4,5-TCP is supported through the marked increase in 3,5-dichlorophenol (3,5-DCP) concentrations. To a lesser extent, the removal of 3,4,5-TCP through 3,5-DCP production was also evident in serum bottles supplied 50 mg COD/L (Figure 4.4). Degradation of 3,4,5-TCP to 3,5-dichlorophenol (3,5-DCP) was slow but apparent in bottles amended with supplemental COD. Limited, production of 3,4-dichlorophenol (3,4-DCP) from 3,4,5-TCP was also observed (Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6). There was no evidence to support the removal of 3,4,5-TCP from the treatment evaluated with only background COD (Figure 4.2).

Table 4.1 Experimental treatment comparison summary

Treatment	Concentration (μm)		% Difference	Max 3,4,5-TCP/Initial PCP
	Initial PCP	Mass Average		
Background	4.043	4.20±0.16	3.9	0.709
10 mg COD/L	3.91	4.38±0.16	12.0	0.931
25 mg COD/L	3.94	4.52±0.23	14.7	0.949
50 mg COD/L	3.70	4.44±0.29	16.6	0.942
100 mg COD/L	3.69	4.29±0.33	13.9	0.937

Initial measurements of chlorophenols at time zero revealed small concentrations of 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) in all serum bottles evaluated. The concentration 2,3,4,6-TeCP in the bottles containing natural COD remained stable over the duration of the duration of the experiment (Figure 4.2). Slight removal of 2,3,4,6-TeCP was observed in all systems evaluated with supplemental COD (Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6). Limited production of 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) from the reductive dechlorination of PCP was observed in the system containing natural COD (Figure 4.2). Conversely, systems supplemented with exogenous COD showed measurable quantities of 2,3,5,6-TeCP production (Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6). Observed production of 2,3,5-trichlorophenol (2,3,5-TCP) in all of the serum bottles, supports removal of 2,3,5,6-TeCP's *ortho* chlorine. Figure 4.7 summarizes the observed metabolites of PCP degradation when imitation vanilla flavoring serves as an electron donor. Where appropriate, solid lines depict observed transformation products. Since the pathway shown in Figure 4.7 was not developed with individual compound degradation tests, alternate pathways are shown by dotted lines.

Evidence of Pentachlorophenol Biotransformation

PCP removal as a function COD treatment in Figure 4.1 displays the difference between serum bottles containing active organisms and those in poisoned controls. In the absence of a viable cell mass, there was no evidence to support abiotic mechanisms of PCP removal. Despite the initial decline in PCP concentrations observed in the first 16 hours of the study, concentrations remained nearly constant at 3.0 μM for the duration of the experiment. Comparing initial and final PCP concentrations over the 519 hour study shows that approximately 15% of the initial PCP mass was removed from the aqueous system. Individual chlorophenols observed in the experimental control are presented with time in Figure 4.8. Concentrations of the chlorophenols observed in the controls showed variability during the first sampling

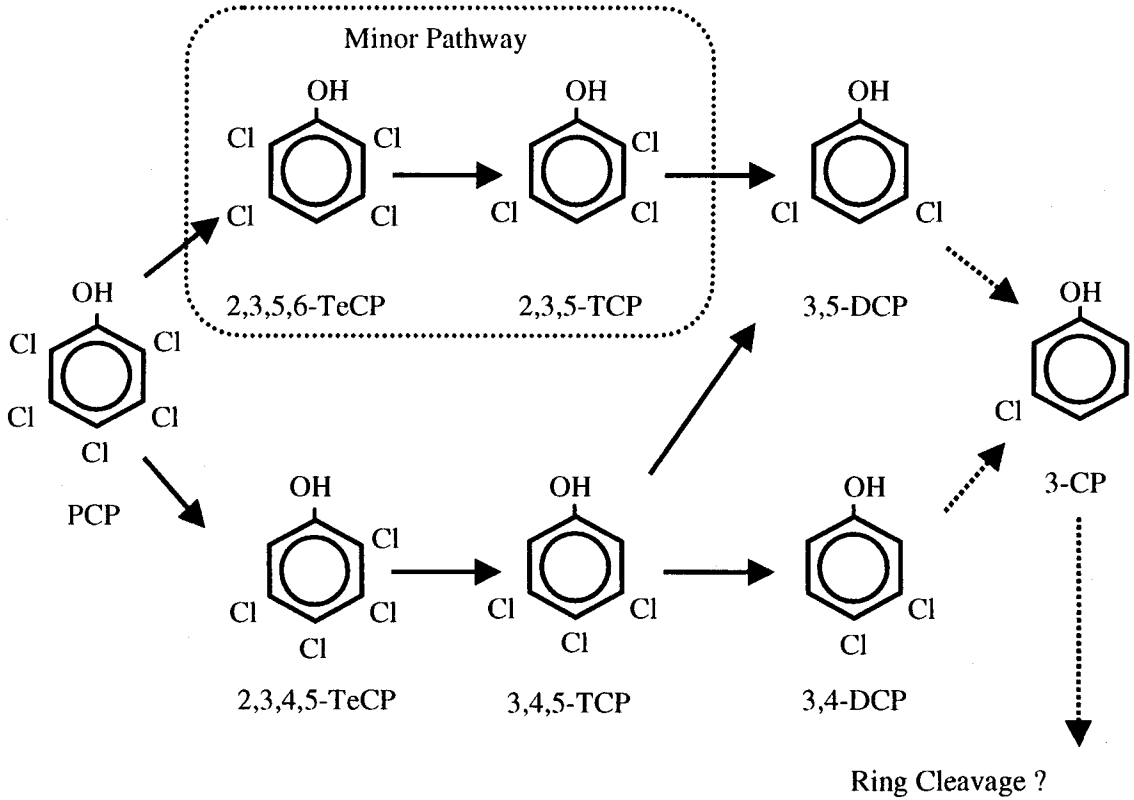

Figure 4.7 Observed experimental PCP reductive dechlorination pathway

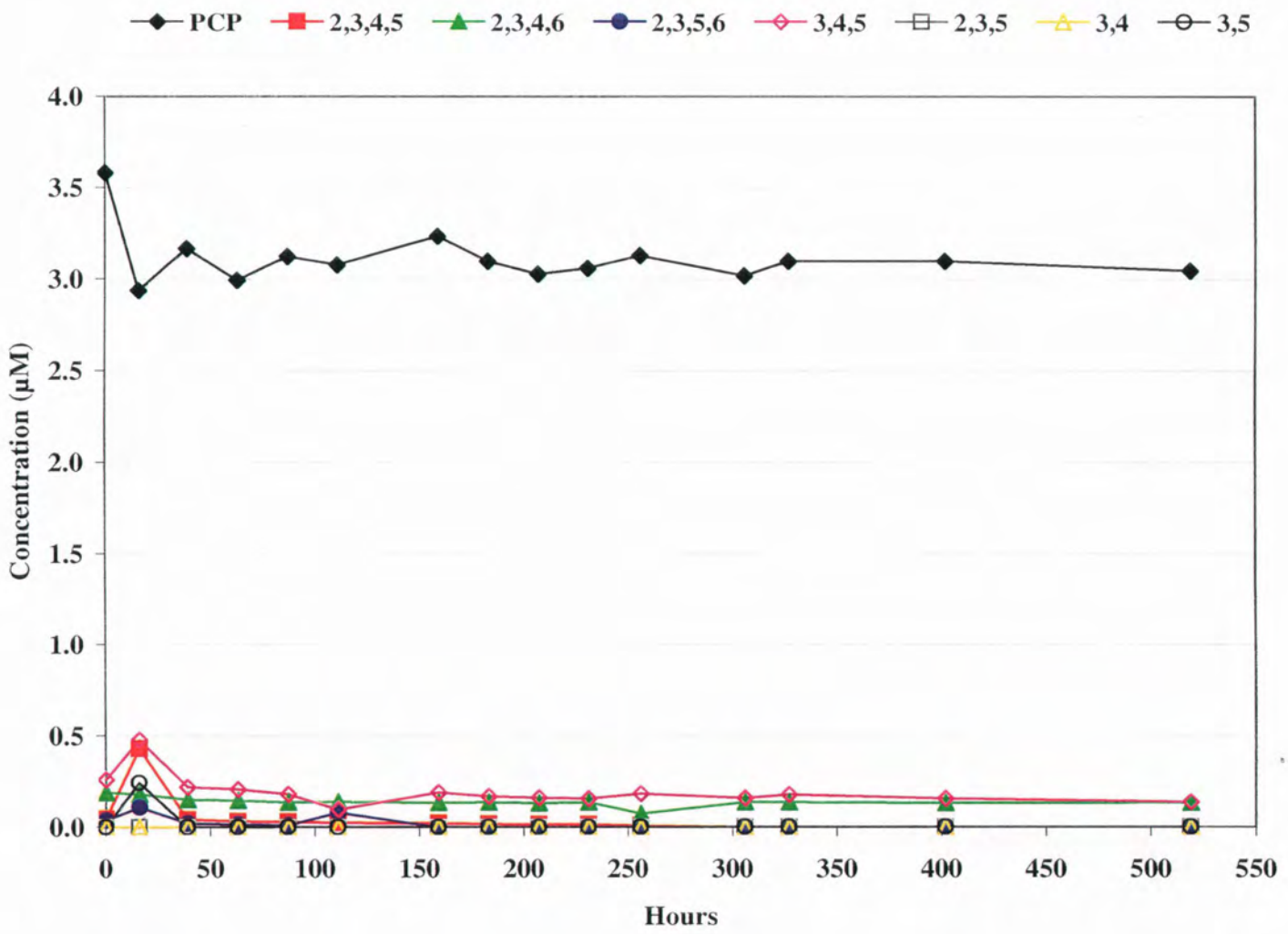

Figure 4.8 Reductive dechlorination pathway observed with experimental controls

period at 16 hours. Periods after 16 hours show stable concentrations for all chlorophenols observed in the control system. The lack of metabolite production in Figure 4.8 is evident when compared to Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6. It is clear that although transformation products are present in Figure 4.8; there is no evidence to support biological or abiotic PCP removal.

RESULTS: FIELD

During the pilot demonstration at the L.D. McFarland facility, PCP degradation was evaluated as a function of electron donor concentration was evaluated. Process and experimental changes imposed on the permeable barrier reactor are summarized in Table 4.2. Imitation vanilla flavoring served as the electron donor for the system. Roman numerals and vertical partitions provide a universal legend for the figures and represent the process changes evaluated and their relative duration. Environmental conditions of the reactor system expressed by apparent E_H and pH are presented with time in Figure 4.9 and Figure 4.10. The apparent oxidation/reduction potential E_H, referenced to the standard hydrogen electrode, is presented in millivolts (mV). Chlorophenol concentrations as function of time during each experimental phase are presented in Figure 4.11 and Figure 4.13. In all figures shown, chlorophenol concentration in micromolar (μM/L) is presented as a function of time in hours. Figure 4.11 and Figure 4.13 include error bars, which represent a confidence interval of 95%. Confidence intervals are based upon the analysis of 28 independent samples collected within the reactor assembly.

Table 4.2 Summary of field conditions evaluated

Phase	Process Conditions	Mixing Conditions	Start	Stop
III	Inoculum, electron donor 100 mg COD/L *Mixed Anaerobic/Aerobic Conditions*	Nitrogen 2 sec./15 min Oxygen 2 sec./15 min	1/14/98	2/27/98
IV	Inoculum, electron donor 100 mg COD/L *Anaerobic Conditions Prevalent*	Nitrogen < 1 sec./15 min Oxygen < 1 sec./15 min	2/27/98	6/6/98
V	Inoculum, electron donor 50 mg COD/L *Anaerobic Conditions Prevalent*	Nitrogen < 1 sec./15 min Oxygen < 1 sec./15 min	6/6/98	12/30/98
VI	Inoculum, electron donor terminated *Anaerobic Conditions Prevalent*	Nitrogen < 1 sec./15 min Oxygen < 1 sec./15 min	1/13/99	3/1/99
VII	Inoculum, electron donor 10 mg COD/L *Anaerobic Conditions Prevalent*	Nitrogen < 1 sec./15 min Oxygen < 1 sec./15 min	3/1/99	5/3/99

Phase III

During phase III, treatment zone A of the reactor was mixed with nitrogen gas while, oxygen was supplied to mix treatment zones B and C. Environmental conditions present during this experimental period are summarized in Figure 4.9-III. Generally, apparent E_H values in both treatment zones indicated aerobic conditions prevailed in the reactor system. Apparent E_H values collected from zone B were typically 150 mV greater than values observed in zone A. Over phase III, pH conditions in treatment zones A and B remained stable; pH values averaged 7.2 and 7.1, respectively. Figure 4.11-III summarizes the response of PCP to the experimental COD treatment of 100 mg/L under an oxidative environment. A gradual decrease in PCP and minor increases in 2,3,4,5-TeCP and 3,4,5-TCP (intermediate product data not shown) concentrations suggested the removal of PCP from the reactor system.

To estimate the magnitude of PCP removal in phase III, the total molar mass of tetrachlorophenols observed in the system was normalized to the PCP concentration observed. Figure 4.12 represents the molar ratio of tetrachlorophenols to PCP during the various experimental phases. Plotted on the right axis is the actual molar concentration of the components. Despite the variability induced by seasonal water table fluctuations, evaluation of the ratio plotted revealed that field concentrations of PCP and lower order chlorinated phenolic compounds were relatively stable through phases I and II. An increase in the ratio at the onset of phase III suggests that the transformation of PCP to tetrachlorophenols was slowly occurring in the reactor or surrounding aquifer structure. Based upon the oxidative conditions observed in the reactor (Figure 4.9) the potential for PCP reductive dechlorination within the reactor system was unlikely.

Phase IV

Environmental conditions present in the treatment system during phase IV are summarized in Figure 4.9-IV. In the reactor system, pH was nearly neutral. Typically, pH in each treatment zone did not fluctuate more than one pH unit; values ranged between 7.3 and 8.3. Apparent E_H measurements in the reactor system varied widely during phase IV. Immediately following the decrease in oxidant supply to the system, the apparent E_H in both treatment zones fell rapidly before eventually stabilizing near –270 mV (Figure 4.9-IV). Apparent E_H measurements during phase IV were relatively stable with the exception of two intermittent increases in redox potential. There was no deviation noted in system operation to account for the observed spikes in the reactor system apparent E_H measurements.

Following changes in the reactor system environmental conditions, evidence of PCP biodegradation was confirmed by the observation of several intermediates of PCP reductive dechlorination. PCP concentrations observed during phase IV are illustrated in Figure 4.11-IV. By the end of phase IV, PCP was removed from an initial system concentration of 4.2 μM to levels below detection. On April 2, a distinct acceleration in PCP degradation was observed. The rate shift occurred among a time frame when all other physical conditions monitored in the reactor assembly remained constant. Under a COD treatment of 100 mg/L, PCP removal within the reactor system approached 100%. Figure 4.11-IV clearly illustrates a

trend of decreasing PCP concentrations with time. Based upon the observed products and their relative distribution in the system, PCP degradation proceeded by reductive dechlorination and followed the pathway presented in Figure 4.7. In the reactor system, PCP degradation proceeded by sequential *ortho* dechlorinations to form 3,4,5-TCP. In turn, 3,4,5-TCP removal was witnessed by the production of 3,5-DCP and to a lesser extent, 3,4-DCP. There was no observed accumulation of 3,5-DCP or 3,4-DCP in the reactor system with time. Routine qualitative analysis was unable to confirm the presence of the potential degradation products, 3-chlorophenol, 4-chlorophenol or phenol in the reactor samples.

Phase V

Degradation studies conducted during phase V centered on a 50% reduction in electron donor supply. Feed rates of the imitation vanilla flavoring were modified to provide 50 mg/L COD to the treatment unit. Environmental conditions present in the reactor during phase V were stable and favorable for biological reductive dechlorination processes (Figure 4.7-V). The mixing frequency and concentration of electron donor supplied (50 mg/L COD) conditions set during phase V remained constant until January 14, 1999 when the electron donor was removed from the system. Degradation of PCP during phase V was nearly 100% (Figure 4.11-V). PCP concentrations in the reactor were routinely measured at or below the detection limit of 0.0038 μM. Figure 4.11-V shows that the transition in electron donor supply had no appreciable effect on the removal efficiencies of PCP in the pilot scale reactor.

Unseasonably cold weather prompted the shut down of the electron donor supply and mixing systems in mid December. Groundwater temperatures were unaffected by the cold weather. For the entire month of December 1999, the operating temperature of the permeable barrier averaged 13.0°C (S.D.=0.156). Despite the change in surface temperature, *in-situ* operating conditions of the permeable barrier reactor were not affected. All surface systems were restarted late in December 1999. Nutrient injection and mixing were reestablished and operated for a period of 15 days before the electron donor supply was halted at the onset of phase VI of the field experiments.

Phase VI

In phase VI, the supply of electron donor to the permeable barrier reactor was suspended. Environmental conditions measured in the reactor system showed no appreciable change in the absence of electron donor supply. Baseline apparent E_H measurements in each treatment zone remained highly reduced while neutral pH conditions prevailed (Figure 4.10-VI). Again, transient spikes in treatment zone apparent E_H measurements were observed despite the fact that no changes were made in reactor operating conditions. Following electron donor suspension, PCP concentrations initially remained constant at values slightly above zero. With increasing time however, the absence of donor addition was noted. PCP concentrations in the reactor unit began to climb slowly above concentrations previously observed in the presence of electron donor. Figure 4.13 depicts reactor performance in phases V, VI and VII. Effective barrier operation in phases V through VII significantly reduced aqueous chlorophenol concentrations in the

system. Therefore, the concentration scale presented in Figure 4.13 was shifted by a factor of ten to plot chlorophenol measurements. Prior to the termination of electron donor, biodegradation of PCP was evaluated under the experimental conditions of phase V (50 mg/L COD). There were no appreciable deviations in reactor performance over the time break between Figure 4.10 and Figure 4.13. While the behavior of PCP during phase VI was erratic, Figure 4.13 clearly shows a change in the performance of the reactor system in the absence of electron donor addition. Historically, in the presence of external electron donor, long term operation of the permeable barrier resulted in PCP concentrations at or below detection levels. The increase in PCP concentrations, observed in the absence of external donor supply, indicates that an exogenous electron donor is required for the complete *in-situ* removal of PCP.

Phase VII

The supply of electron donor was returned to the reactor in phase VII. Environmental conditions present during this phase are shown in Figure 4.10-VII. There was little change in the reactor pH following the addition of electron donor (10 mg/L COD). Conditions in the reactor unit remained stable during the phase transition. Apparent E_H measurements varied widely at the onset of electron donor addition. Elevated redox measurements followed the experimental transition as an artifact of phase VI conditions rather than a result of electron donor addition during phase VII. Transient spikes in apparent E_H measurements, again were observed without apparent cause. With increasing time, apparent E_H measurements in the reactor stabilized near –270 mV, which corresponds to the baseline condition observed for experiments conducted during phases V-VI.

Following the observed increase in PCP concentrations in the reactor system, electron donor was returned to provide a concentration of 10 mg/L COD within the treatment unit. PCP concentrations during phase VII, shown in Figure 4.13-VII, generally decreased with increasing time. Throughout phase VII, PCP concentrations in the reactor continued to decrease. Chlorophenol removal approached 100 % in the reactor system with observed concentrations of PCP and it's metabolic products at or below limits of analytical detection. Removal of PCP was favored in the presence of electron donor addition. Based upon the variation in PCP concentrations observed in the transition from phase VI to VII, it was impossible to identify where PCP reductive dechlorination commenced as a direct result of electron donor addition.

DISCUSSION

Comparison of field and laboratory results illustrates the importance for the determination of an electron donor concentration capable of supporting PCP reductive dechlorination. Laboratory studies conducted in controlled serum bottle assays indicated that PCP reductive dechlorination was attainable in all COD treatments investigated. While the natural COD of the site groundwater allowed for reductive dechlorination, bottles amended with 10-100 mg COD/L showed higher efficiency in PCP removal.

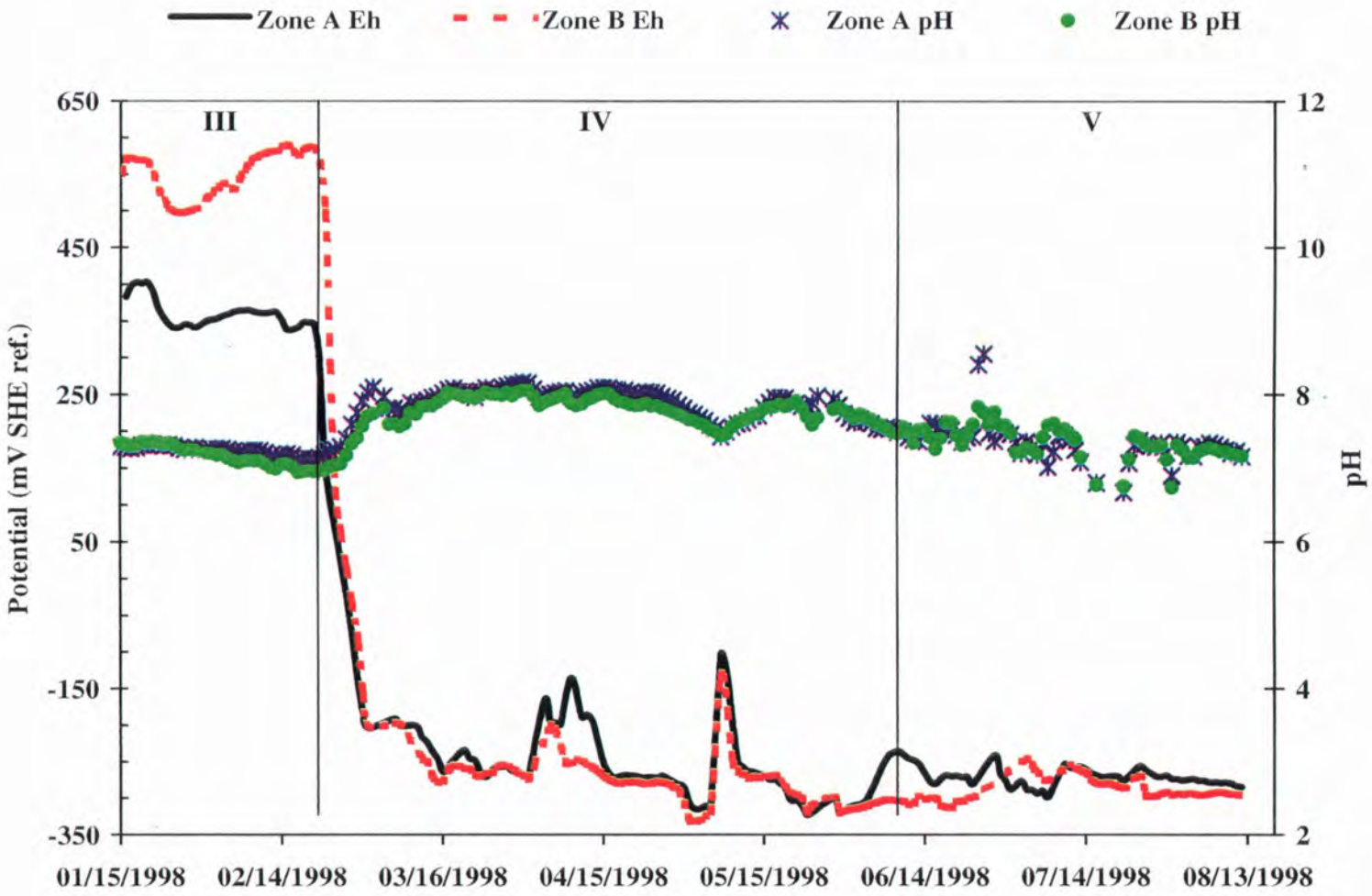

Figure 4.9 Environmental conditions observed in-situ during phases III – V

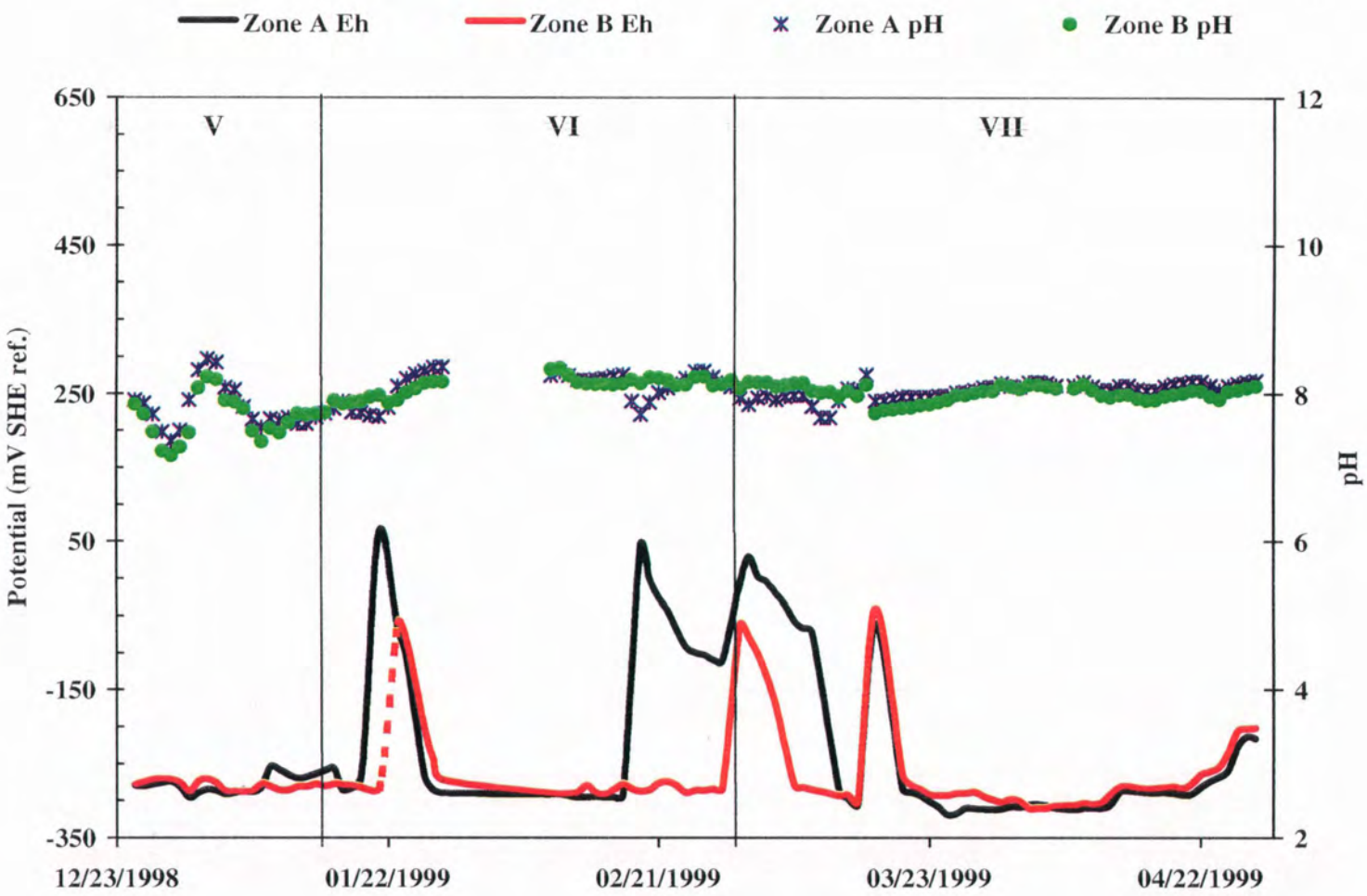

Figure 4.10 Environmental conditions observed in-situ during phases V – VII

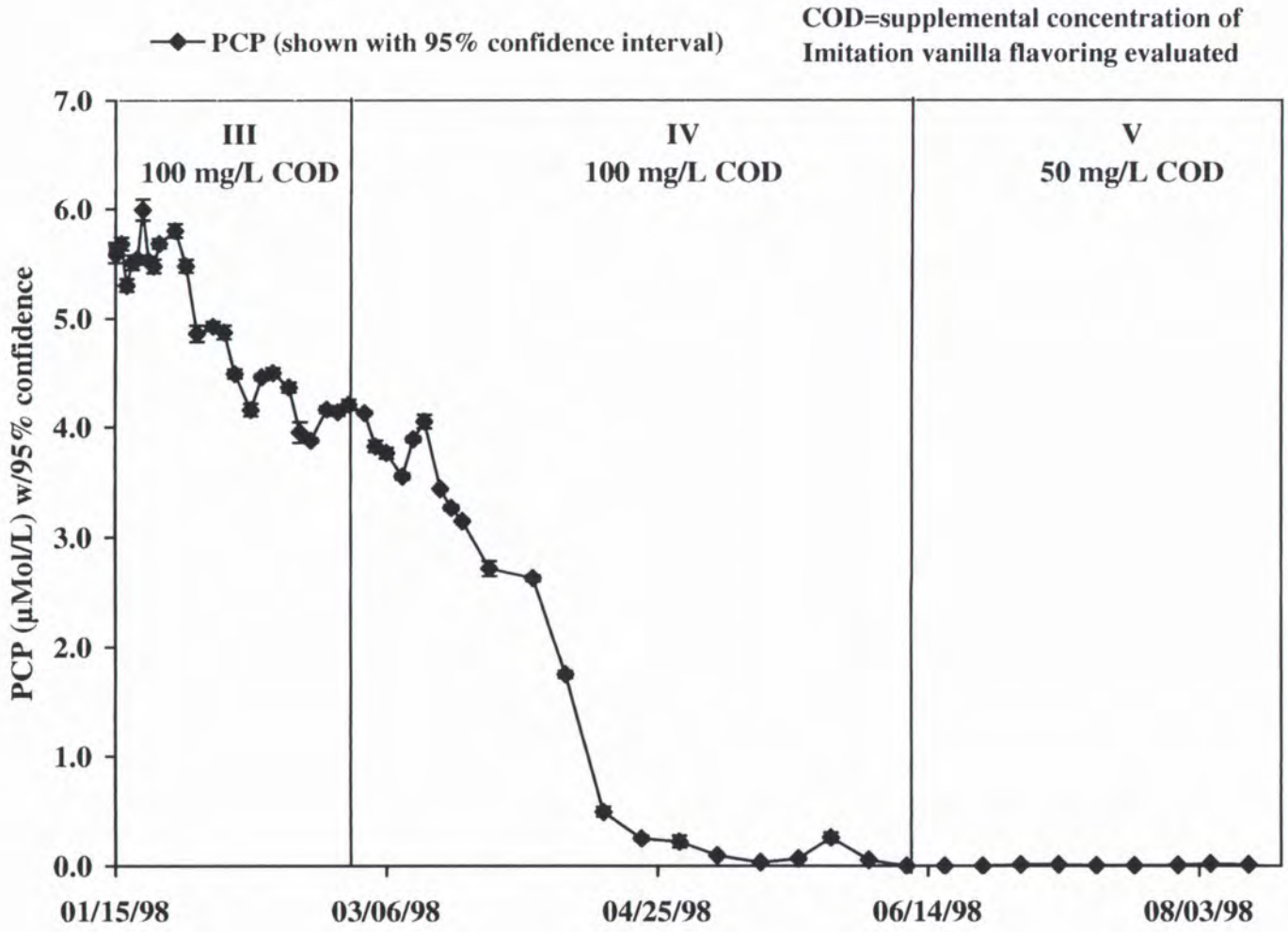

Figure 4.11 Pentachlorophenol concentrations observed in-situ during phases III - V

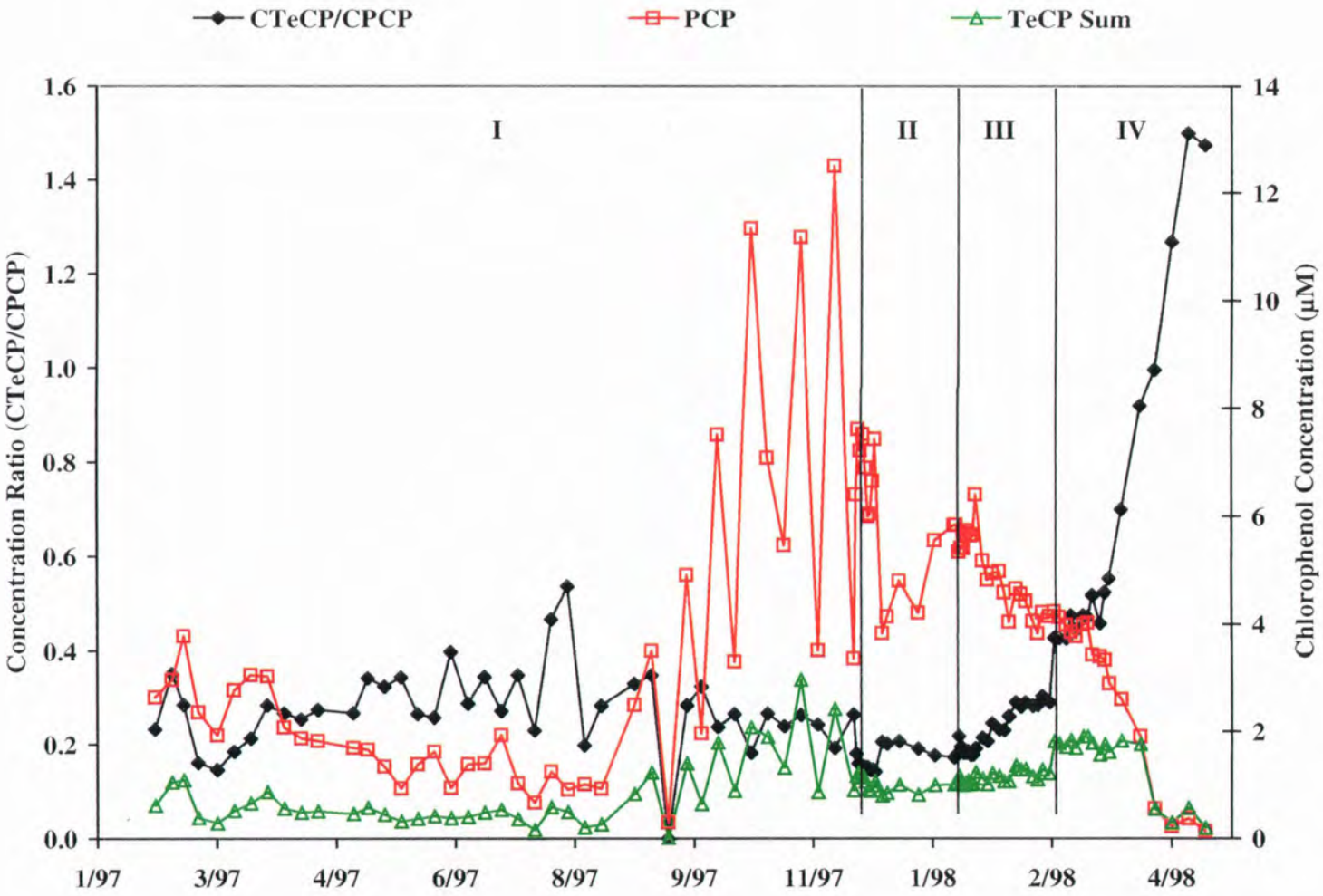

Figure 4.12 Concentration ratio of TeCP/PCP observed during phases I-IV

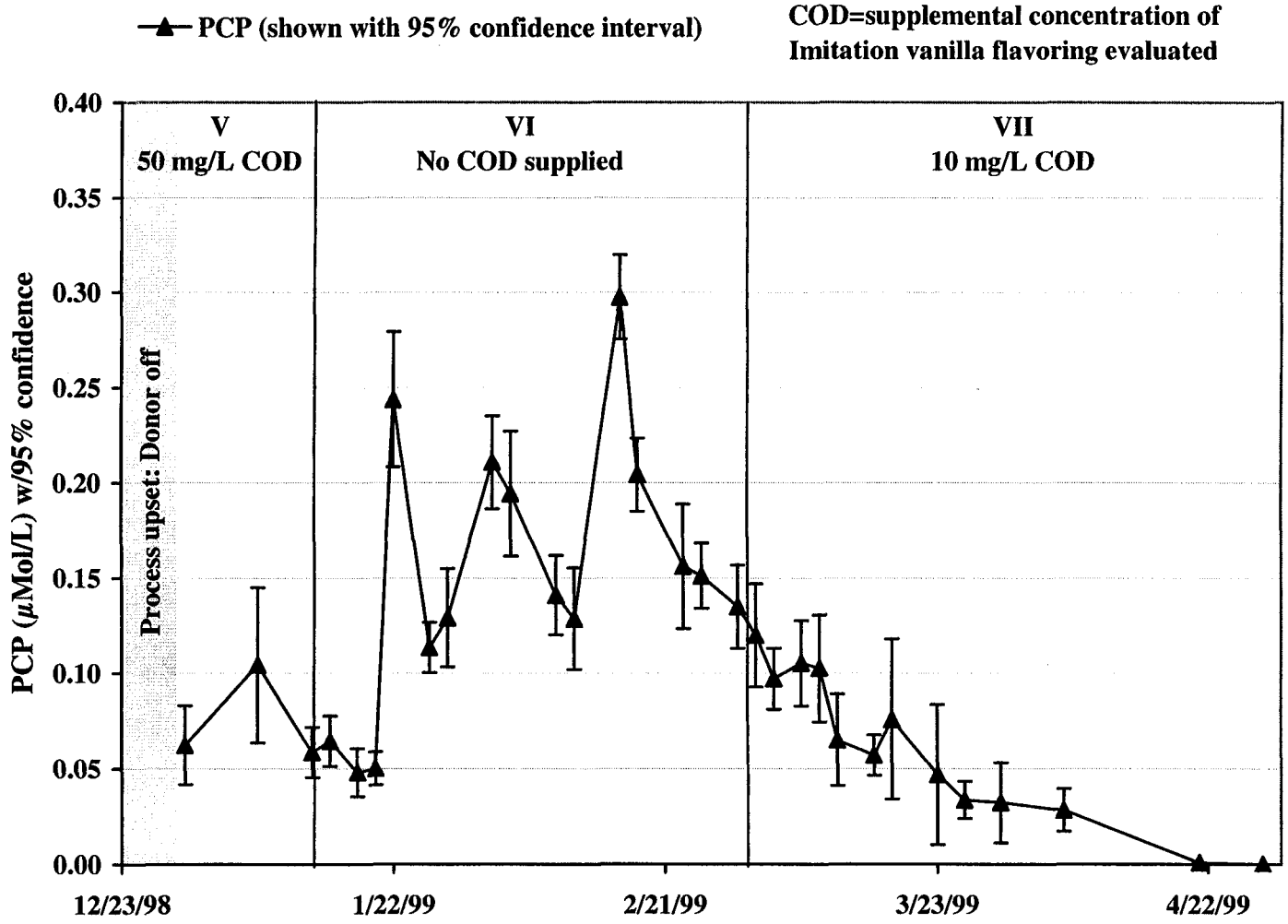

Figure 4.13 Pentachlorophenol concentrations observed in-situ during phases V - VII

The Effect of Electron Donor Concentration

PCP reductive dechlorination was supported by an endogenous electron donor in treatments lacking supplemental COD. However, the addition of supplemental electron donor clearly accelerated the removal rate of PCP (Figure 4.1). Surprisingly in systems provided supplemental COD at 10 or 100 mg/L, there was no appreciable difference in the rate of PCP reductive dechlorination observed.

Prior the installation of the permeable barrier reactor, natural *in-situ* transformation of PCP was not observed. Therefore, the ability of the consortium to degrade PCP in the absence of electron donor addition under natural background COD was unexpected. Similar performance of field and laboratory systems evaluated under natural COD indicate there was a sufficient quantity of biologically available carbonaceous materials to support PCP degradation *in-situ*. The ability for the field system to support PCP transformation in the absence of exogenous donor supply may be directly related to the quantity of biomass developed over the previous year of reactor operation. It is possible that soluble organic products present in the cell mass were able to sustain PCP degradation in the absence of donor supply.

When the individual performance of each serum bottle is compared, subtle differences are apparent in the production and ultimate removal of 2,3,4,5-TeCP. In systems lacking supplemental COD (Figure 4,2), immediate production and accumulation of 2,3,4,5-TeCP is evident. Incomplete transformation of 2,3,4,5-TeCP occurred. Conversely, in all systems in which supplemental COD was supplied (Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6) biologically produced 2,3,4,5-TeCP was immediately consumed. Complete removal of 2,3,4,5-TeCP was observed. Like the observed degradation of PCP, in bottles systems supplemented with exogenous COD, there were no obvious differences in the production and removal rates of 2,3,4,5-TeCP among the various COD treatments evaluated. Findings of the laboratory studies support similar studies which showed nearly equivalent rates of PCP degradation over a range of COD loading rates (Khodadoust et al., 1997; Wu et al., 1993). Under sulfate reducing and methanogenic environments no appreciable change was observed in chloroform transformation rates when supplemental donor concentrations, acetate, were slowly increased from 50 to 200 mg/L. A distinct difference was observed between the rate of chloroform transformation in systems evaluated in absence and presence of 50 mg/L acetate (Gupta et al., 1996a; Gupta et al., 1996b).

The systems evaluated under natural background COD were not truly representative of an experimental condition in which an electron donor was completely absent. Construction of a true optimization study to determine minimum threshold concentrations requisite for PCP reductive dechlorination was neither feasible nor desired. The physical setting of the field demonstration made it impossible to create experimental conditions to evaluate the minimum required donor concentrations. The companion field and laboratory experiments were conducted to evaluate the benefit, if any achieved when the supply of exogenous electron donor was reduced and eventually terminated. The overall goal was simply to determine the quantity of donor required to sustain anaerobic PCP transformation.

Laboratory PCP degradation under varying COD treatments supported observations made in the field (Figure 4.13-VI). Like observations made in parallel serum bottles, natural background COD present in the reactor system was capable of supporting PCP degradation. Results indicated there was a clear difference between the rate and extent of degradation between background COD and the lowest supplemental donor concentration evaluated (10 mg/L COD). Under the lowest substrate concentration evaluated effective PCP transformation occurred. Fulfillment of the experimental goal came not in a specific quantity of exogenous donor required but rather in the finding that the system could be operated without loss of degradation efficiency following a 90% reduction in electron donor concentration.

The lack of system response to increases in electron donor concentration may be explained by the fact that all concentrations of supplemental donor evaluated were in excess. Therefore, increases in concentration would have no effect on the rate of PCP reductive dechlorination. While this conclusion concisely explains the observed performance of the serum bottle system, it is possible that chemical complexity of the substrate evaluated controlled the rate of PCP degradation. The chemical composition of imitation vanilla flavoring contains three substituted aromatic structures and propylene glycol. It is possible that PCP degradation was solely supported through the anaerobic oxidation of propylene glycol. However, to harness the full reducing power of the electron donor, oxidation of the aromatic structures would be required. Degradation of the electron donor, imitation vanilla flavoring, was not specifically investigated. Degradation however, likely occurs through a fermentative pathway. In the presence of the anaerobic consortium, soluble compounds are fermented to organic acids by the acetogenic community. Oxidation of the organic acids by members of the consortium results in the release of hydrogen, which is effectively consumed by hydrogenotrophic organisms.

While the role of the supplemental electron donor in the anaerobic system evaluated is unknown, many have speculated that the microbial mechanisms of reductive dechlorination are supported by hydrogen (DiStefano et al., 1992; Maymo-Gatell, 1995; Mohn and Kennedy, 1992). If hydrogen was required for reductive dechlorination in the system evaluated fermentation of the supplemental donor to organic acids or hydrogen may be the rate-limiting step for PCP degradation. This conclusion would also support the results of the study: independence of electron donor concentration on the rate of PCP reductive dechlorination. Clearly, this conclusion requires further investigation. Degradation studies of the individual components of the donor mixture may provide needed scientific support a relationship between biologically derived hydrogen and PCP reductive dechlorination.

Contribution of Alternate Electron Donors

In addition to background COD, PCP degradation in the field may have been sustained by the sheer mass of organisms present in the reactor system. Conversely, an alternate electron donor may also have fueled field degradation processes.

Site groundwater analysis has always shown soluble COD present in the aquifer structure at the L.D. McFarland Facility. Depending on the time year, COD measurements in the aquifer structure vary from 5 to 50 mg/L. Background COD is believed to originate from components present in the aromatic oil which was used as a carrier solvent for wood preservation with PCP.

It is possible that organisms used to inoculate the permeable barrier reactor have developed mechanisms to oxidize dissolved components of the treating oil to provide an electron flow suitable for PCP reduction. Anaerobic oxidation of petroleum hydrocarbons may result in the production of aromatic, aliphatic and alicyclic organic acids, phenols and aldehydes (Cozzarelli et al., 1995). Ultimately, these metabolic products of hydrocarbon transformation could serve as potential electron donors for the microbial community responsible for PCP reductive dechlorination. While not specifically shown to support reductive dechlorination, anaerobic transformation of toluene has been shown with several terminal electron acceptors: denitrifying (Hutchins, 1993), iron reducing (Lovley and Lonergan, 1990), sulfate reducing (Edwards et al., 1992) and methanogenic (Grbic-Galic and Vogel, 1987). Naphthalene, a known component of the treating oil has been shown to degrade *in-situ* under sulfate reducing conditions (Thierrin et al., 1993).

In the anaerobic environment, hydrogen, a versatile electron donor, is generally produced through the fermentation of organic compounds. However, hydrogen evolution from the anaerobic corrosion of iron has been shown to support methanogenesis (Lorowitz et al., 1992), reductive dechlorination of chlorinated solvents (Matheson and Tratnyek, 1994) and pesticides (Sayles et al., 1997) or the reduction of nitroaromatic compounds (Heijman et al., 1995). The reactor system was installed and operated in a large diameter well casing constructed of carbon steel. The availability of iron from interior surfaces of the casing coupled with the reducing environment present in the reactor system (Figure 4.9 and Figure 4.10), produced ideal conditions for hydrogen generation by the reduction of water. In light of the hypotheses presented however, it remains unclear what mechanisms were truly responsible for the observed degradation phenomena.

Observed Degradation Pathway

While results from the laboratory study supported the donor concentration evaluations in the field, the extent of PCP degradation differed dramatically in each system evaluated. In all COD treatments studied with the serum bottles, PCP reductive dechlorination proceeded through sequential reductions at the *ortho* position (Figure 4.7). With the exception of 100 mg COD/L treatment (Figure 4.7), the primary product of PCP degradation, 3,4,5-trichlorophenol (3,4,5-TCP) accumulated in all serum bottles studied (Figure 4.2 and Figure 4.4). In contrast, under each of the donor conditions evaluated in the field study, complete removal of PCP and intermediate degradation products was observed. It may be possible that 3,4,5-TCP accumulation in the serum bottles resulted from the exhaustion of reductant source. However, this idea fails to provide reason for the similarity in PCP degradation rates when compared as a function of

supplemental electron donor concentrations. Perhaps the disparity observed in the extent of PCP degradation is a function of physical system evaluated. Whereby, an element vital to 3,4,5-TCP removal was depleted in the batch serum bottles while, operation of the *in-situ* reactor allowed for continual replacement from the surrounding aquifer material. The possibility of an alternate electron donor present only in the aquifer may also have significantly contributed to the differences observed in the extent of PCP degradation.

Experimental Controls

The construction of a parallel control for experiments conducted in the field was not feasible. However, the performance of poisoned controls, was evaluated parallel to the laboratory serum bottle study (Figure 4.1). In comparison to active serum bottles in the presence and absence of supplemental electron donor addition, PCP concentrations in the control system were constant over the duration of the study. There was no evidence to support biological PCP removal in the control system evaluated. Furthermore, the concentrations or metabolic products of PCP degradation, present with the inoculum, remained unchanged (Figure 4.8). When the performance of the active bottle systems (Figure 4.2, Figure 4.3, Figure 4.4 Figure 4.5 and Figure 4.6) is compared to that of the poisoned controls (Figure 4.8), it is clear that PCP was transformed by biologically mediated reductive dechlorination.

SUMMARY AND CONCLUSIONS

It seems logical to believe that increasing supplemental electron donor concentrations could result in higher transformation rates of PCP. Results from this study proved otherwise. It is clear from both the laboratory and field data that the reductive dechlorination of PCP in the system investigated requires very little supplemental COD. The performance of serum bottle containing 10 mg COD/L was nearly equal to an identical system with an initial COD ten times greater. Serum bottle results indicate that electron donor concentrations in a comparable system could be reduced by a factor of ten without compromising rates of PCP reductive dechlorination. Serum bottle predictions were supported by the operation of the pilot reactor in the field. When supplied 10 or 100 mg/L supplemental COD, there was no noticeable difference in PCP degradation. However, suspension of electron donor supply resulted in an increase in PCP concentrations in the *in-situ* permeable barrier. While the electron donor concentrations were not truly optimized, study results showed the operation of the unit was identical when supplied 10 mg COD/L or 100 mg COD/L.

The contribution of alternate electron donors for PCP reductive dechlorination seems likely. Based upon the excellent performance of the serum bottle evaluated without supplemental COD, the pool of natural donor seems quite large. Operation of the pilot scale reactor in the absence of supplemental COD injection supported the observations made in the serum bottle; PCP transformation was possible without the supply of an external electron donor. PCP was degraded in the serum bottles and in the biological

permeable barrier by the process of reductive dechlorination. Degradation of PCP in the laboratory proceeded by the sequential reduction of PCP's *ortho* chlorine atoms to yield 3,4,5-TCP, which accumulated. In systems supplied 50 and 100 mg COD/L, there was evidence to support further transformation of 3,4,5-TCP to 3,5 DCP. In contrast, operation of the biological permeable barrier in the field resulted in the complete removal of PCP. No accumulation of intermediate degradation products was observed.

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CHAPTER 5

THE EFFECT OF SULFATE ON THE REDUCTIVE DECHLORINATION OF PENTACHLOROPHENOL: A FIELD AND LABORATORY COMPARISON

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ABSTRACT

The effect of sulfate on the reductive dechlorination of pentachlorophenol (PCP) was evaluated under field and laboratory conditions. PCP degradation in the presence of sulfate, a competitive electron acceptor was examined under laboratory conditions using batch serum bottles incubated at 14°C and amended with 10 mg/L supplemental COD. Sulfate was supplied to the serum bottles at initial concentrations of 78, 156, 312, 781 and 2604 μM (7.5, 15, 30, 75 and 250 mg/L). Serum bottle assays were conducted in duplicate and parallel poisoned controls were constructed to assess abiotic PCP degradation. The presence of sulfate was not inhibitory to PCP degradation. However, compared to systems evaluated in the absence of sulfate, slower rates of PCP transformation were observed. Sulfate reduction was not appreciable in laboratory serum bottles. In laboratory studies, the pathway of PCP degradation was independent of sulfate concentration investigated. Under laboratory conditions, PCP was degraded to 3,4,5-trichlorophenol (3,4,5-TCP) by sequential carbon reduction in PCP's *ortho* positions. Concentrations of 3,4,5-TCP accumulated in the serum bottles. There was evidence to support transformation of 3,4,5-TCP to 3,5-dichlorophenol and 3,4-dichlorophenol in serum bottles evaluated with 75 and 250 mg/L sulfate. *In-situ* degradation studies were conducted in a PCP-contaminated aquifer using a biological permeable barrier reactor. Results from the field and laboratory studies were complementary. Approximately 10 mg/L supplemental carbonaceous oxygen demand (COD) from imitation vanilla flavoring was supplied as the electron donor. A sodium sulfate solution was continuously injected into the reactor system to provide a calculated sulfate concentration of 100 mg/L. Changes in reactor operation and performance were evaluated as a function of time and sulfate injection conditions. Calculation of field sulfate concentrations should have placed initial sulfate concentrations near 100 mg/L. Yet, during the study the maximum observed sulfate concentrations were approximately 20 mg/L. *In-situ* sulfate reduction was presumed responsible for the discrepancy in the sulfate concentrations observed in the field. Under field conditions, PCP degradation in the presence of exogenous sulfate was complete. No accumulation of intermediate products was observed in the permeable barrier reactor. Findings of this study indicate that a biological based treatment system for the remediation of PCP-contaminated groundwater could effectively be deployed in an aquifer structure that contains sulfate.

RESEARCH OBJECTIVES

Reductive dechlorination is a biologically catalyzed oxidation/reduction reaction where the chlorinated compound, acting as an electron acceptor, is reduced. Like all redox reactions, electron flow is generated through the oxidation of an electron donor. In anaerobic environments where reductive dechlorination is favored, terminal electron acceptors like sulfate compete for available reductant. In the application of an anaerobic treatment regime for chlorinated groundwater contaminants, the effectiveness of reductive dechlorination may be compromised by a population competing for available donor for sulfate reduction.

Therefore, this study was designed to estimate the feasibility of biological treatment strategies for chlorinated compounds in groundwater systems containing sulfate. Anaerobic pentachlorophenol degradation in the presence of a competitive electron acceptor, sulfate, was evaluated under laboratory and field conditions. Field based experiments were conducted at the pilot scale using a permeable barrier reactor. The reactor assembly was fabricated to fit within the casing of a large diameter well that was constructed in a PCP-contaminated aquifer at the L.D. McFarland facility in Eugene, Oregon. Specifically, this field and laboratory comparison study was undertaken with the following objectives:

- Determine the effect of sulfate on the reductive dechlorination of PCP under field and laboratory conditions.
- Evaluate the pathway of PCP reductive dechlorination under field and laboratory conditions in the presence and absence of a competitive electron acceptor.
- Investigate the feasibility for anaerobic biological treatment strategies for the remediation of groundwater containing chloroaromatics and sulfate.

INTRODUCTION

As a groundwater remediation strategy, the use of biological treatment techniques shows great promise for the mineralization of xenobiotic compounds. Application of *in-situ* biological treatment processes require an understanding of the complex relationships that exist among the contaminant, microorganisms and physical conditions present in subsurface environment. Environmental conditions such as groundwater temperature, velocity, oxidation/reduction (redox) potential, and geochemical speciation often dictate the success of *in situ* bioremediation efforts. Effective operation of an *in-situ* bioremediation scheme, therefore, requires an understanding of the interactions between environmental conditions and the microbial population mediating transformation or mineralization of the target contaminant.

In the application of an *in-situ* aerobic treatment regime, the delivery of oxygen to a subsurface population continues to present a formidable engineering challenge. While the effectiveness of subsurface oxygen delivery methods continues to improve, mass transfer and mixing limitations hinder the effectiveness of *in-situ* aerobic processes. Therefore, the application of a biological process that functions with electron acceptors other than oxygen offers some distinct advantages. In anaerobic groundwater systems, the oxidation of anthropogenic or natural organic material is often coupled to the reduction of terminal electron acceptors like nitrate, iron (III), sulfate and carbon dioxide. Unlike oxygen, these terminal electron acceptors are often naturally present in the groundwater formation. Land use practices, soil composition and aquifer recharge sources all contribute to the relative abundance of these electron acceptors in the subsurface. The participation of an electron acceptor in a redox reaction catalyzed by microorganisms is largely dependent upon the compounds physical properties and the mediating organisms.

Table 5.1 displays important natural redox couples as a function of their standard reduction potentials and mediating organisms.

Generally, electron transport drives the redox reaction while; the relative abundance of the donor, acceptor, and products often determines the thermodynamic feasibility. Sequential reduction of terminal acceptors follow a thermodynamic hierarchy that predicts electron acceptors with the highest oxidation-reduction potential (redox) will be reduced first (Zehnder and Stumm, 1988). The redox conditions of a particular environment play an integral role in selecting the electron donor-acceptor couple used in a redox reaction (Brock and Madigan, 1991). Therefore, the microbial community may be dictated by the group of organisms that can most effectively consume the electron acceptor with the highest redox potential. This principle plays a very important role in the maintenance of subsurface environmental conditions as it defines the basis of competition for electron donor among the various microbial groups. The reduction of a particular acceptor relative to the external environmental conditions provides a basis from which, inferences about a biological population can be drawn (Table 5.1).

Table 5.1 Standard reduction potential (E_0) for selected electron acceptors

Half Reaction	Mediating Organisms	E_0 (mV)	Reference
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	Aerobes	+ 820	1
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	Iron Reducers	+ 760	1
$NO^-_3 + 2H^+ + 2e^- \rightarrow NO^-_2 + H_2O$	Denitrifyers	+ 420	1
$PCP + H^+ + 2e^- \rightarrow$ (see Table 5.2)	Reductive Dechlorinators	$400 \leftrightarrow 290$	2
$SO_4^{-2} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$	Sulfate Reducing Bacteria	- 220	1
$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$	Methanogens	- 240	1
$2CO_2 + 8H^+ + 8e^- \rightarrow CH_3COOH + 2H_2O$	Acetogens	- 290	1
$2H^+ + 2e^- \rightarrow H_2$	-	- 420	1

Reference Notes:

1. Adopted from (Brock and Madigan, 1991)
2. Adopted from (Dolfing and Harrison, 1992)

The competition for electron donor among nitrate reducing bacteria, sulfate reducing bacteria and methanogens was shown with hydrogen in a pristine aquifer structure (Lovley et al., 1994). In a contaminated aquifer, the redox hierarchy may control the microbial mechanisms by which xenobiotic

aerobic respiration of aromatic fuel components resulted in anoxic conditions through the consumption of dissolved oxygen. Anaerobic respiration with nitrate and iron (III) followed until exhaustion of terminal electron acceptors in the groundwater system led to donor competition between methanogens and sulfate reducers (Cozzarelli et al., 1995; Thierrin et al., 1993; Vroblesky et al., 1996).

Hydrogen plays an important role in controlling the external conditions present in the anaerobic environment. Whether biologically-derived through fermentation or volatile acid degradation or produced abiotically thorough metal corrosion hydrogen, can support the electron donor requirements for many members of the anaerobic community (Brock and Madigan, 1991; Lorowitz et al., 1992). As such, competition exists among the many species of organisms that have developed mechanisms to use hydrogen as an electron donor. In anaerobic systems where methanogens and sulfate reducers directly compete for reductant, the sulfate reducers are generally more successful at sequestering hydrogen than methanogens. The ability to out compete organisms for hydrogen is directly related to the sulfate reducers half velocity coefficient (K_s) which is significantly lower than the K_s of the methanogens. The lower K_s of the sulfate reducing bacteria provide a distinct advantage in hydrogen competition over the methanogens especially at lower hydrogen concentrations (Kristjansson et al., 1982; Robinson and Tiedje, 1984). At unit activity, thermodynamics predict higher energy yields for sulfate reduction than for methanogenesis when either hydrogen or acetate serve as the electron donor (Karhadkar et al., 1987). Several groups have also reported that sulfate reducers have the ability to out compete methanogens at low hydrogen levels (Lovely, 1985; Lovely et al., 1982). Others have shown concurrent sulfate reduction and methanogenesis when growth was not limited by substrate availability (Achtnich et al., 1995; Maillacheruvu et al., 1993; Uberoi and Bhattacharya, 1995; Vroblesky et al., 1996). Studies of aquifer hydrogen concentrations and geochemical speciation conducted by Lovley et al., 1994 corroborate with the ability of sulfate reducers to thrive at hydrogen levels lower than those required for methanogenesis.

Transformation of chlorinated phenols by reductive dechlorination occurs in biologically mediated redox reactions whereby the phenolic compound participates as the electron acceptor (Mohn and Tiedje, 1992). Therefore, organisms capable of chlorophenol reduction compete among the mediating groups listed in Table 5.1 for available electron donor. Table 5.2 summarizes the standard reduction potentials for two commonly observed pathways of PCP reductive dechlorination. Standard reduction potentials provide a reference of the reactions feasibility in the environment. Ultimately, the reaction that takes place is one in which the dominant organism gains the greatest benefit. In the presence of multiple terminal electron acceptors, competition for electron donor among the anaerobic community may provide insight to factors that contribute to incomplete transformation of chlorinated organic compounds.

Table 5.2 Standard reduction potential (E_0) for phenolic electron acceptors (Dolfing and Harrison, 1992)

Half Reaction	E_0 (mV)	Abbreviations
PCP + H^+ + $2e^-$ → 2,3,5,6-TeCP + Cl^-	+ 446	TeCP-tetrachlorophenol
PCP + H^+ + $2e^-$ → 2,3,4,5-TeCP + Cl^-	+ 399	
2,3,5,6-TeCP + H^+ + $2e^-$ → 2,3,5-TCP + Cl^-	+ 337	TCP-trichlorophenol
2,3,4,5-TeCP + H^+ + $2e^-$ → 3,4,5-TCP + Cl^-	+ 316	
2,3,5-TCP + H^+ + $2e^-$ → 3,5-DCP + Cl^-	+ 393	DCP-dichlorophenol
3,4,5-TCP + H^+ + $2e^-$ → 3,5-DCP + Cl^-	+ 395	
3,5-DCP + H^+ + $2e^-$ → 3-CP + Cl^-	+ 290	MCP-monochlorophenol
3-MCP + H^+ + $2e^-$ → phenol + Cl^-	+ 418	

There is growing evidence to support the hypothesis that hydrogen serves as the electron donor for the reductive dechlorination of chlorinated aromatic and aliphatic compounds. Mohn and Tiedje, 1992 report an obligate anaerobe *Desulfomonile tiedjeii* that uses hydrogen as an electron donor in the reductive dechlorination of 3-chlorobenzoate. Similar findings by DiStefano et al., 1992 and Maymo-Gatell, 1995, report the capacity of mixed and enrichment cultures capable of tetrachloroethene reductive dechlorination using hydrogen as an electron donor. The ability of hydrogen to serve the needs of a population capable of reductive dechlorination has interesting implications in the application of *in-situ* anaerobic treatment processes. The ability of hydrogen to support dechlorinators, sulfate reducing bacteria and methanogens creates a unique situation where each microbial group is in competition for the same electron donor. The potential then exists where the anaerobic transformation of chlorophenols are partially or completely inhibited by the inability of the dechlorinating population to channel electrons away from competing processes.

The reductive dechlorination of pentachlorophenol (PCP) has been shown to proceed under both sulfate reducing and methanogenic conditions. Chlorophenol reductive dechlorination under sulfate reducing conditions has been successful demonstrated (Häggblom and Young, 1990). The studies were conducted with estuarine sediments as an inoculum. Transformation of sulfate to sulfide was observed in the absence of methanogenesis. Addition of molybdate completely inhibited sulfate reduction and chlorophenol transformations. Madsen and Aamand, 1991 examined the effect of sulfate on PCP degradation by a methanogenic culture derived from sewage sludge. Results showed the reductive

dechlorination of PCP under methanogenic conditions. However, under the sulfate reducing conditions PCP degradation was inhibited. Following the addition of molybdate, a specific inhibitor of sulfate reduction, PCP reductive dechlorination rates were comparable to the methanogenic system evaluated. Hydrogen competition among dechlorinators and sulfate reducers was suggested to explain the poor removal of PCP observed in the sulfate system. Many research groups have evaluated the effects of sulfate on the reductive dechlorination of chlorophenols; results are summarized in Table 5.3.

In general, the presence of sulfate inhibits reductive dechlorination. Conversely, several groups have reported the reductive dechlorination of PCP and other chlorophenols in the presence of sulfate (Table 5.3). In cases where sulfate inhibited reductive dechlorination, a common conclusion was presented by two independent authors; dechlorinating species could not compete with the sulfate reducing bacteria at the hydrogen levels present (Häggblom and Young, 1990; Zhang and Wiegel, 1990). Overall, it seems that estuarine sediments were more effective at chlorophenol degradation and showed less sulfate inhibition than other culture sources. While the reason for the apparent performance difference is unknown, it could be related to the organisms ability to tolerate sulfate which was likely present in the initial environment.

Reductive dechlorination remains one of the most effective processes for the microbial degradation of highly halogenated aromatic compounds. The ability of a natural electron acceptor like sulfate to inhibit or alter the degradation process has serious implications for the ultimate success of *in-situ* biological treatment schemes. To evaluate the effect of sulfate on PCP reductive dechlorination, companion laboratory and field studies were conducted. In the field, PCP degradation in a pilot scale permeable barrier was evaluated in the presence and absence of sulfate. The pilot reactor is housed within the casing of a large diameter well installed in a PCP-contaminated aquifer in Eugene, Oregon. Details of the reactor construction, operation and site characterization have been provided elsewhere (Cole and Woods, 2000b). Specifically, this study was undertaken to determine the effect of a competitive electron acceptor, sulfate on the *in-situ* reductive dechlorination of PCP and to estimate the feasibility of biological treatment strategies for chlorinated compounds in sulfate rich groundwater systems.

Table 5.3 The Effect of sulfate and nitrate on the reductive dechlorination of pentachlorophenol

Inoculum	State[1]	Cmp[2]	Acceptor	Results[3]	Reference
Aquifer[4]	SR	CPs	SO_4^{2-}	R.D after SO_4^{2-} degradation	(Gibson and Suflita, 1986)
Estuarine[4]	SR	DBP	SO_4^{2-}	R.D before SO_4^{2-} degradation	(King, 1988)
Aquatic[4]	M	CPs	SO_4^{2-}	R.D inhibited by SO_4^{2-}	(Sharak Genthner et al., 1989)
Estuarine[4]	M,SR	CPs	SO_4^{2-}	R.D no inhibition	(Häggblom and Young, 1990)
Consortium	M	PCP	SO_4^{2-}	R.D inhibited by SO_4^{2-}	(Madsen and Aamand, 1991)
Anaerobe		TCP	SO_4^{2-}, NO_3^{1-}	R.D no inhibition; NO_3^{1-} reduction	(Madsen and Licht, 1992)
Consortium	M	PCP	SO_4^{2-}, NO_3^{1-}	R.D inhibited by SO_4^{2-}; R.D inhibited by NO_3^{1-}	(Häggblom et al., 1993a)
Estuarine[4]	SR	PCP	SO_4^{2-}	R.D No inhibition	(Masunaga et al., 1996)
Estuarine[4]	SR	PCP	SO_4^{2-}	R.D inhibited by SO_4^{2-}	(Liu et al., 1996)
Consortium	M	PCP	SO_4^{2-}	R.D inhibited by SO_4^{2-}; R.D inhibited by NO_3^{1-}	(Juteau et al., 1995b)
Soil	M	PCP	SO_4^{2-}, NO_3^{1-}	R.D enhanced by SO_4^{2-}; inhibited by NO_3^{1-}	(Chang et al., 1996)
Consortium	M	PCP	SO_4^{2-}, NO_3^{1-}	R.D inhibited by SO_4^{2-}; R.D inhibited by NO_3^{1-}	(Chang et al., 1998a)

Notes:
1. The culture state evaluated: SR = Sulfate Reducing, M = Methanogenic
2. Compound type investigated: CP-several chlorophenols, DBP-Dibromophenol, TCP-Trichlorophenol
3. R.D = Reductive Dehalogenation
4. 4.Origin of Sediments used

MATERIALS AND METHODS

Anaerobic pentachlorophenol degradation in the presence of a competitive electron acceptor, sulfate, was evaluated under laboratory and field conditions. Field based experiments were conducted at pilot scale using a custom designed permeable barrier reactor. The reactor assembly was fabricated to fit within the

casing of a large diameter well that was constructed in a PCP-contaminated aquifer at the L.D. McFarland facility in Eugene, Oregon. Details of the reactor and the supporting control systems are outlined in Chapter 3 and Appendix B through Appendix F. To evaluate PCP degradation in the presence of a competitive electron acceptor, sodium sulfate was injected into the permeable barrier reactor. In the field, imitation vanilla flavoring was supplied as an electron donor and PCP degradation was evaluated in the absence or presence of sulfate addition. Laboratory studies were conducted in batch serum bottles that had previously been used to evaluate PCP degradation as a function of supplemental electron donor concentrations. Degradation of PCP in the presence of five different sulfate concentrations was evaluated in duplicate. Parallel controls were used to discern abiotic and biological PCP removal. Progress curves constructed over the experiment duration were used to monitor the effect of sulfate on chlorophenol degradation rates and pathways. Selected components of the imitation vanilla were also monitored to ensure the systems were not electron donor limited. Finally, gas production in the active and control bottles was measured on a volumetric basis.

Laboratory Experimental System

Glass 300 ml serum bottles were used to conduct the degradation study. Each bottle contained 45 ml of headspace and 255 ml of liquid. Bottles were screw capped with Teflon® faced butyl rubber stoppers. Following the termination of a previous serum bottle study, which examined the effect of electron donor upon PCP degradation, cells present in ten active serum bottle were settled and decanted. In the confines of an anaerobic glove box, 200 ml of liquid was removed and replaced with freshly collected site ground water. To each pair of serum bottles, an aqueous, oxygen free, solution of PCP and sodium sulfate was added to provide an initial PCP concentration of 4.0 μM. The serum bottle sets were constructed at room temperature over a range of initial sulfate concentrations (78, 156, 312, 781 and 2604 μM). A uniform addition of an electron donor, imitation vanilla flavoring was made in all active bottles to provide supplemental COD of 10 mg/L.

Serum bottle preparation for each sulfate concentration evaluated was identical: 40 ml inoculum, 200 ml groundwater, 15 ml PCP stock, 100 μl concentrated imitation vanilla flavoring stock and an appropriate mass of sodium sulfate were added to the serum bottles. Controls were constructed in open air: using 235 ml sterilized groundwater, 15 ml PCP stock and imitation vanilla flavoring to provide 50 mg COD/L. To match experimental conditions, sodium sulfate was added to provide a sulfate concentration of 885 μM. In addition to sterilization, controls were chemically poisoned with mercuric chloride. The bottles were capped, shaken and immediately sampled for initial concentrations of sulfate and PCP. The serum bottles were removed from the glove box and purged with nitrogen to flush the headspace of hydrogen captured during bottle closure in the anaerobic chamber. Purge gas was stripped of residual oxygen by in line contact with copper filings at 450°C. Serum bottles were incubated in the dark at 14±2°C; the median of observed site aquifer temperatures.

Inoculum

Consortia used in the serum bottle assay were originally harvested from the pilot scale permeable barrier reactor system installed at the L.D. McFarland facility in Eugene, Oregon. The organisms were used in a previous laboratory study and results indicated a viable population of PCP degrading organisms. Site groundwater used in the serum bottle study was pumped from a central location in the permeable barrier reactor and dispensed to a 4-liter vessel that was continuously purged with nitrogen gas. The vessel was sealed and transported on ice to the laboratory. Upon arrival in the laboratory, site groundwater was immediately used for construction of the serum bottle pairs.

Field Experimental System

A detailed description of operation and control of the mixing and nutrient supply system in the permeable barrier reactor is found in Chapter 3 and Appendix B through Appendix F. In field based experiments, a mixture of imitation vanilla flavoring and sodium sulfate were continuously pumped to the reactor mixing zones. A common 10-L reservoir supplied two FMI QG-6 positive displacement pumps, Fluid Metering Inc. (Oyster Bay, NY) which dispensed the feed mixture to the reactor through 1/8" O.D. (3.2 mm) Teflon® tubing. Standard size gas cylinders and two-stage regulators were used to supply low pressure mixing gas to the diffuser assemblies. Mass flow of the mixing gases was controlled by two adjustable electric solenoid valves Cole-Parmer® (Vernon Hills, IL). Under field conditions, supplemental COD from donor addition was held constant at 10 mg/L; two feed concentrations of sodium sulfate were injected (50 and 200 g/L).

Laboratory Sampling Procedure

The serum bottles and experimental controls were sampled at room temperature with time to monitor chlorophenol and sulfate concentrations. Gas production was measured with a 5 ml luer tip syringe (Popper & Sons, New Hyde Park, NY). The syringe walls were first lubricated with de-ionized water to allow easy plunger movement. Air present in the syringe barrel was expelled and a new syringe needle was attached. The serum bottle septa were then punctured with the syringe. Displacement of the plunger indicated gas production since the last sampling interval. Chlorophenol samples were collected from the bottles with 100 µl syringes (Hamilton Co., Reno, NV) and were immediately prepared for analysis. Sulfate samples were collected with serum bottle dedicated 1 ml polypropylene syringes. Samples were dispensed to polypropylene eppendorf centrifuge tubes. Prior to anion analysis, samples were centrifuged at 10,000 RPM for 10 minutes.

Field Sampling Procedure

Using the pneumatic sampling principle previously outlined in Chapter 3 aqueous samples were collected from the permeable barrier reactor with time. Samples were collected from all locations in the reactor and dispensed into 4 ml amber vials with Teflon® faced screw caps. Samples were stored on ice for

transport to the analytical laboratory. Field samples were analyzed for chlorophenol concentration upon laboratory arrival. Field measurements of nitrate, sulfate and ferric iron were made using anion/cation specific colorimetric Hach Accuvac® test kits, Hach (Loveland, CO). A portable colorimeter, (Hach DR 890) was used to analyze, interpret and store collected field data.

Chemical Sources

Pentachlorophenol (purity > 99.9%) was obtained from Sigma Chemical Co. (St. Louis, MO) and was used without further purification. Individual components of the imitation vanilla flavoring were obtained from Aldrich Chemical Co. (Milwaukee, WI). All were reagent grade and possessed purity greater than 99%. Sodium sulfate was obtained from Mallinckrodt Co., (Paris, KY). The analytical internal standard, 2,4,6-tribromophenol and authentic chlorophenol congeners were obtained from Ultra Scientific Inc., (North Kingston, RI).

Analytical Procedures

Aqueous chlorophenol samples were acetylated, extracted into hexane and analyzed as derivatives by capillary gas chromatography. The method was a modification of procedure developed by ((NCASI), 1981; Voss et al., 1980). Extractions were conducted as follows: 500 µl of a solution containing 30.4 g/L K_2CO_3 and 250 µg/L 2,4,6 tribromophenol was combined with a 100 µl chlorophenol sample in a disposable glass culture tube with a Teflon® faced cap. 100 µl of acetic anhydride was added and the tube was mechanically shaken for 20 minutes. 1 ml of chromatographic grade hexane was added and the tube was shaken for an additional 20 minutes. Hexane was removed from the tube and transferred to a 2 ml amber glass vial. The vial was sealed with a Viton® faced crimp cap. Vials were immediately loaded for chromatographic analysis.

Chlorophenols were quantified on a Hewlett Packard 6890 gas chromatograph. Automated 1 µl injections were made on the inlet, which was operated, in a splitless configuration. A Hewlett Packard Chemstation handled signal acquisition and processing from the 63Ni Electron Capture Detector (ECD). Separation of chlorophenol congeners was accomplished on a J & W Scientific (Folsom, CA) DB-5 fused silica capillary column (30m x 320µm I.D. x 0.25µm film). Helium at 35 cm/s served as the column carrier gas. The inlet temperature was 250°C while; the detector was operated at 350°C. A 95/5 blend of argon/methane supplied at 75 ml/min was used for detector make-up. The instrument was operated as follows: initial temperature of 45°C was held for 2 minutes; the temperature was then increased 25°C/min to 140°C and held for 5 minutes; the oven was then increased 5°C/min to 245°C where it was held for 10 minutes. Sulfate measurement in the serum bottle test was accomplished with a Dionex 2000I ion chromatograph. Field and laboratory measurements COD were conducted using a dichromate digestion procedure. Two-ml samples were placed in commercially prepared tubes, Hach (Loveland, CO) and digested at 150°C for two hours. Sample measurement followed using a Hach DR 890 colorimeter.

RESULTS: LABORATORY

The effect of sulfate on the reductive dechlorination of PCP was evaluated in serum bottles under laboratory conditions. Sulfate was supplied in excess of background through the addition of sodium sulfate to yield concentrations of 78, 156, 312, 781 and 2604 μM (7.5, 15, 30, 75 and 250 mg/L). The concentration of sulfate tested in the serum bottles was selected as a function of the theoretical electron demand required for sulfate reduction to sulfide. Electrons available in the system were assumed to originate only from the oxidation of the electron donor, imitation vanilla flavoring. Assuming each component of the imitation vanilla flavoring was completely oxidized to carbon dioxide, the total electrons available for sulfate and PCP reduction was computed. Each serum bottle constructed contained the same initial concentration of electron donor, imitation vanilla flavoring. Therefore, the same number of reducing electrons was theoretically available in each bottle. Ignoring the electron demand of PCP, sulfate concentrations for each serum bottle were computed to provide theoretical electron demands of 0.5, 1.0, 2.0, 5.0 and 16.7 times the available reducing electrons. The theoretical electron demand (0.5, 1.0, 2.0, 5.0 and 16.7) translated to initial sulfate concentrations of 78, 156, 312, 781 and 2604 μM, respectively. The efficiency of PCP reductive dechlorination in the presence of sulfate was evaluated and compared to experimental observations of PCP degradation in the absence of sulfate. Sterile controls were constructed with sulfate to evaluate the potential of abiotic PCP degradation. Performance of duplicate serum bottles was nearly identical.

Effect of Sulfate on Pentachlorophenol Degradation

The effect of sulfate on PCP degradation at electron demand conditions of 0.5, 1, 2.0, 5.0 and 16.7 are summarized in Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.4, and Figure 5.5, respectively. PCP degradation is plotted as function of time in the absence and presence of sulfate. Table 5.4 provides a summary of PCP transformation in the presence and absence of sulfate. At the lowest sulfate concentration investigated (Figure 5.1), PCP and sulfate were removed concurrently from initial values of 4.2 μM and 97 μM, respectively. Approximately 55% of the initial sulfate present was removed. Degradation in the sulfate amended system preceded slowly while; sulfate free systems showed a faster relative PCP transformation rate. At roughly the midpoint of the time course study, hour 207, only 43% of the initial PCP present in the system was transformed. Comparatively, 62% of initial PCP present in sulfate free serum bottles was transformed by hour 207. In the absence of sulfate, PCP removal measured 84%. Conversely, in the presence of sulfate, PCP transformation measured only 67%. Regardless of initial sulfate conditions, the complete transformation of PCP removal was not observed.

PCP was transformed in the presence of sulfate at 1 times the supplied reducing equivalents (Figure 5.2). Sulfate removal in the system was moderate; concentrations decreased approximately 30% from the initial measured conditions. In comparison to the system evaluated in the absence of sulfate, the relative rate of PCP degradation was significantly slower. At hour 207, 34% and 80% of the initial PCP mass was

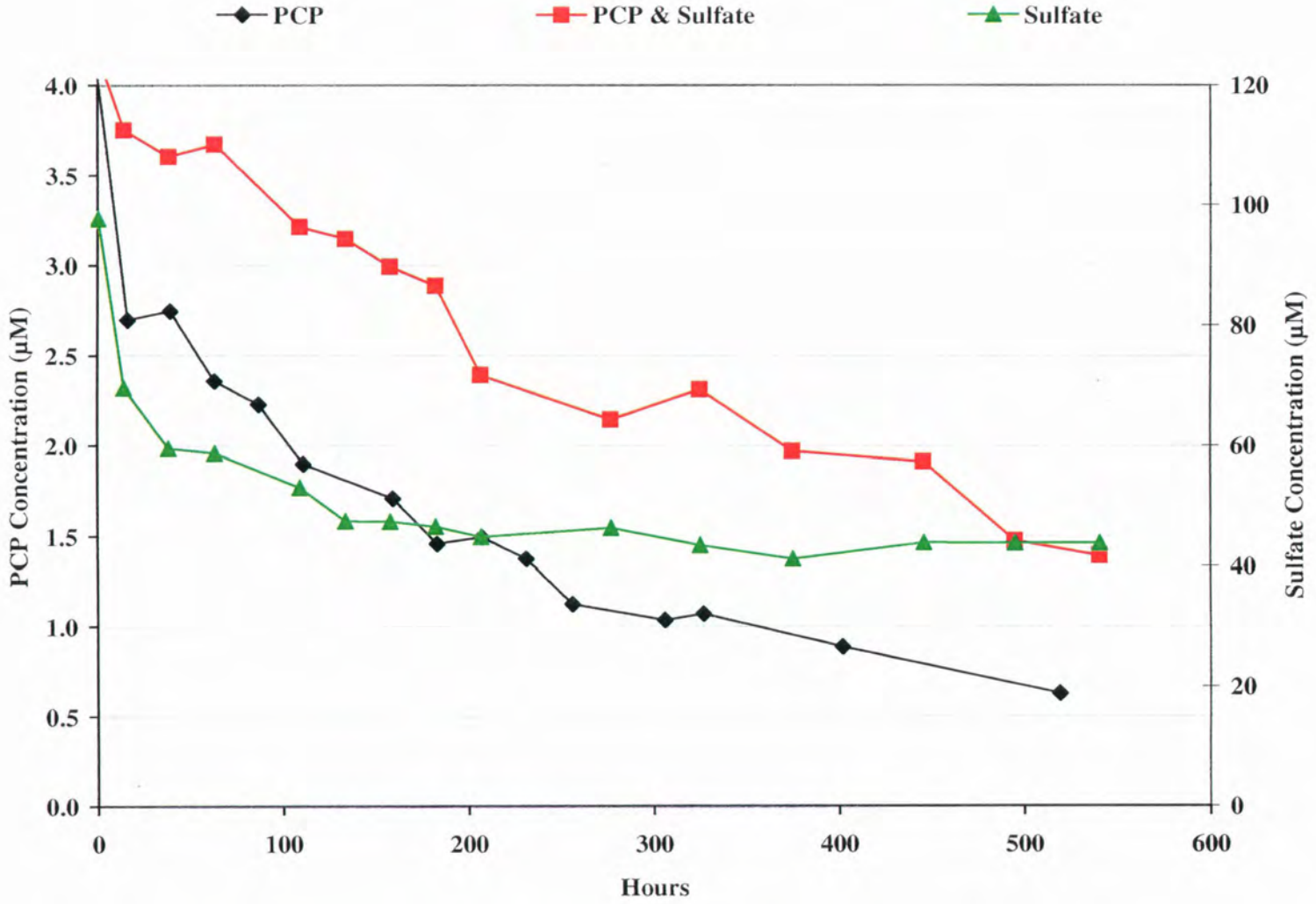

Figure 5.1 The Effect of sulfate on PCP degradation at 0.5x supplied reducing equivalents

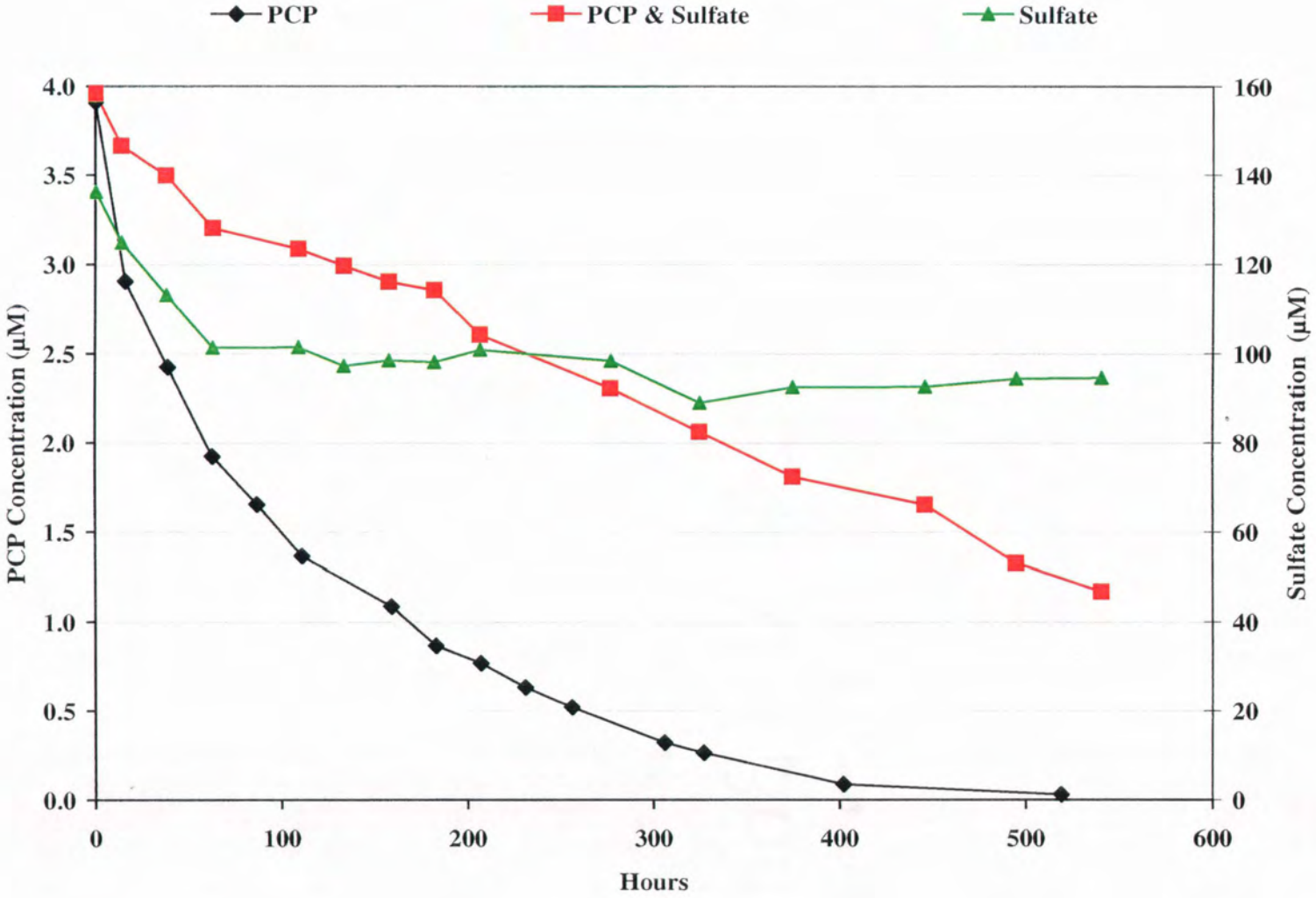

Figure 5.2 The effect of sulfate on PCP degradation at 1x supplied reducing equivalents

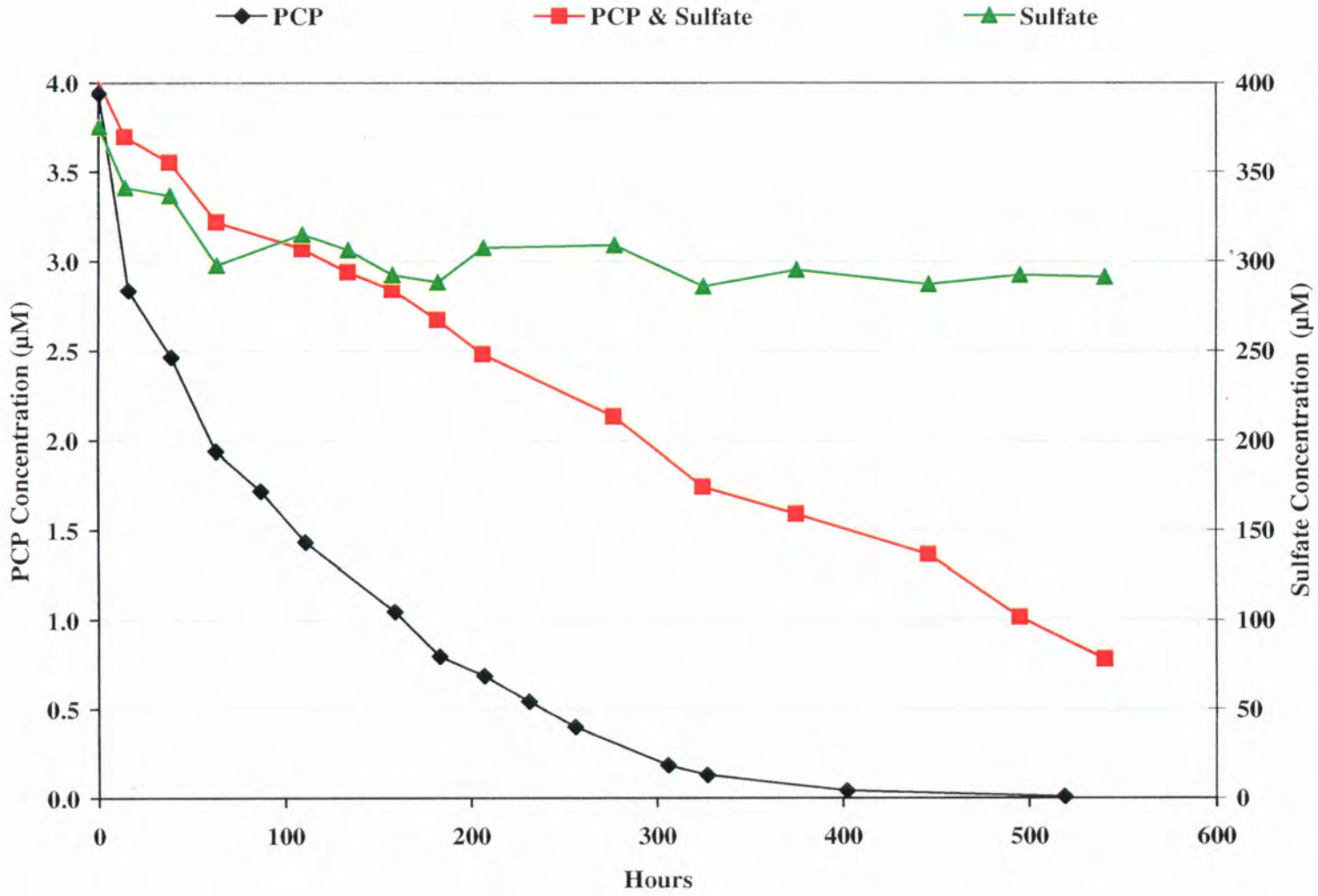

Figure 5.3 The effect of sulfate on PCP degradation at 2x supplied reducing equivalents

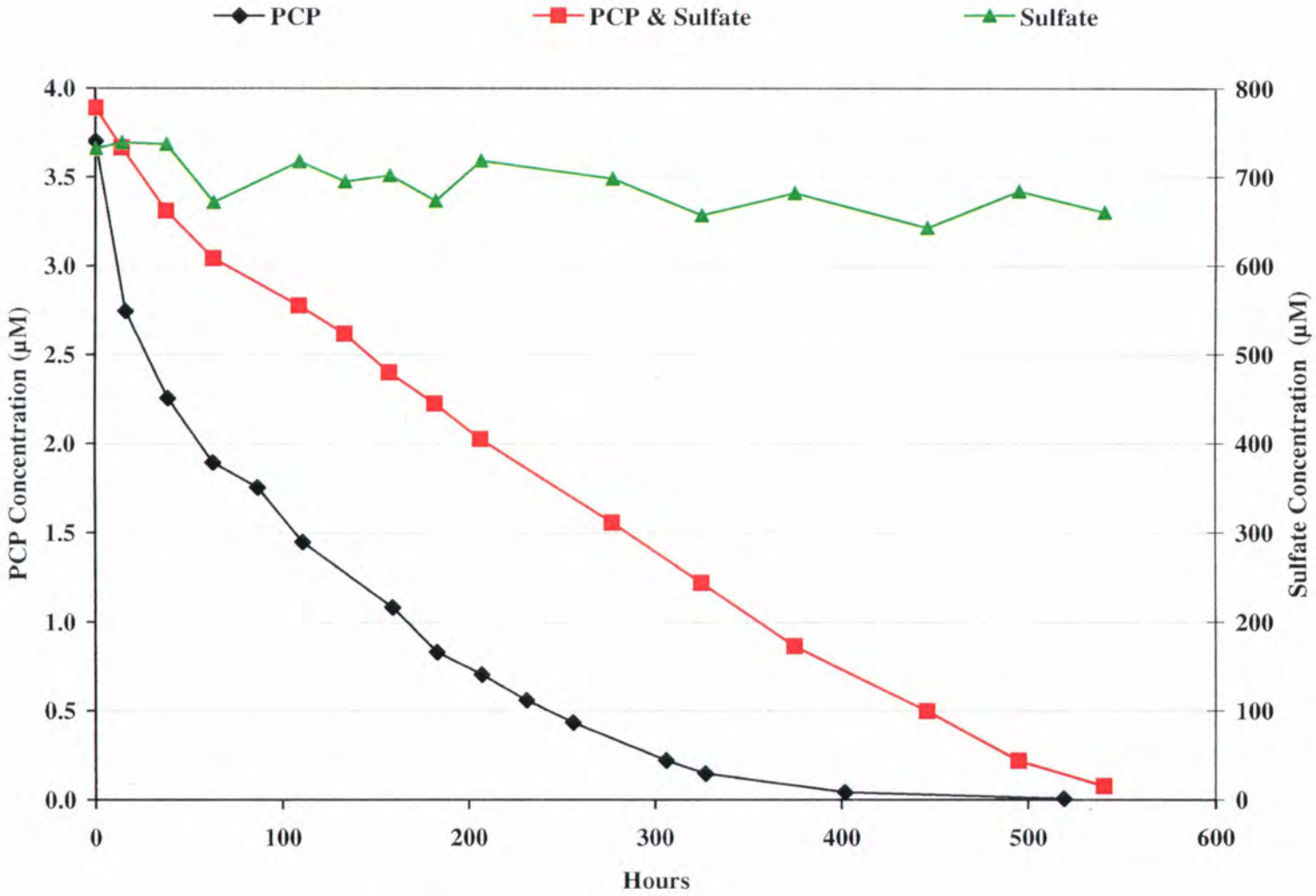

Figure 5.4 The effect of sulfate on PCP degradation at 5.0x supplied reducing equivalents

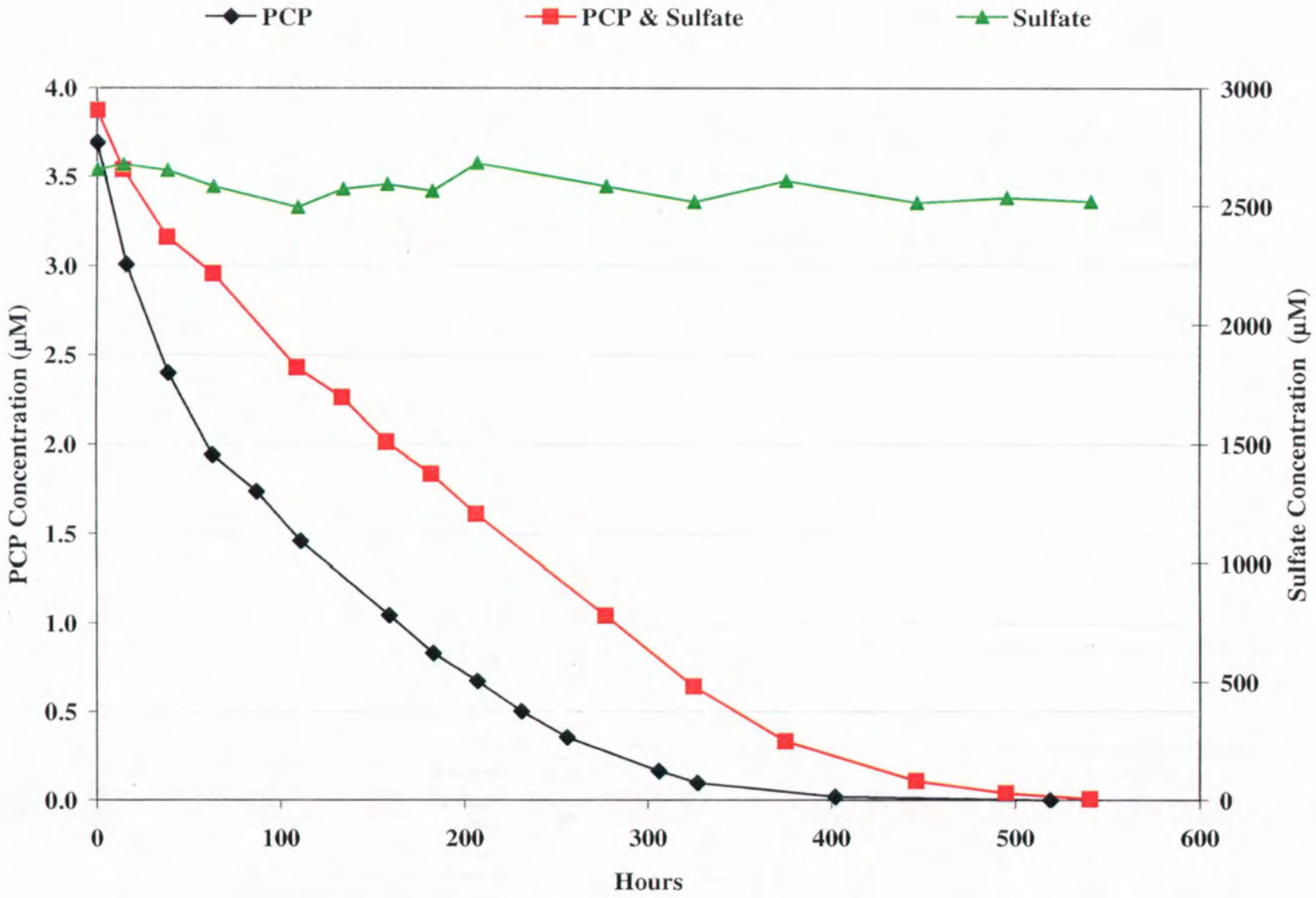

Figure 5.5 The effect of sulfate on PCP degradation at 16.7x supplied reducing equivalents

transformed in sulfate amended and non-amended systems. Transformation of PCP under sulfate free conditions occurred in an exponential fashion whereas, sulfate amended systems followed a more linear pattern. At study termination, systems with or without sulfate contained trace quantities of PCP. Sulfate amended systems effectively transformed 71% of the initial PCP mass. Transformation in the absence of sulfate measured 99.2%.

Despite roughly two times the sulfate concentration, PCP transformation at 2 times the supplied reducing equivalents (Figure 5.3) was nearly identical to systems evaluated with 1 time the reducing equivalents. Approximately 22 % of the initial sulfate present was removed during the course of the study. PCP degradation in the presence and absence of sulfate supported previous observations; in the presence of sulfate, the relative rate of PCP degradation was significantly slower. System comparisons at hour 207, revealed that 38% and 82% of the initial PCP mass was transformed in sulfate amended and non-amended systems, respectively. At study termination, systems with or without sulfate contained trace quantities of PCP. Sulfate amended systems effectively transformed 80% of the initial PCP mass whereas transformation in the absence of sulfate measured 99.8%.

At 5 times the supplied reducing equivalents (Figure 5.4), PCP transformation in the presence of sulfate was observed. Sulfate removal in the system was small; concentrations decreased approximately 10% from the initial measured conditions. In comparison to the system evaluated in the absence of sulfate, the relative rate of PCP degradation was significantly slower. At hour 207, 48% and 81% of the initial PCP mass was transformed in sulfate amended and non-amended systems. Transformation of PCP under sulfate free conditions occurred in an exponential fashion whereas, sulfate amended systems followed a more linear pattern. At study termination, systems with or without sulfate contained trace quantities of PCP. Overall, PCP transformation efficiencies in each system were high. Sulfate amended systems effectively transformed 98% of the initial PCP mass. Transformation in the absence of sulfate measured 99.7%.

Under the highest sulfate loading evaluated Figure 5.5, efficient but incomplete PCP degradation was observed. No appreciable loss of sulfate was noted; concentrations at experiment termination were within 5% of initial measurements. Differences in PCP transformation rates in the sulfate and non-sulfate systems were less pronounced. However, transformation efficiency in the sulfate free systems was noticeably faster. PCP removal from initial conditions at hour 207 of the study measured 59% in the presence and 82% in the absence of sulfate. PCP degradation was complete in sulfate free serum bottles. Overall, 99.8% of the initial PCP mass was removed in the serum bottles containing approximately 2600 μM sulfate.

Table 5.4 PCP degradation performance comparison in the presence and absence of sulfate

Sulfate Amended @T_{zero}			Sulfate Free @T_{zero}	%PCP Removed @$T_{207\ hours}$		%PCP Removed @$T_{540\ hours}$	
e^- Demand	PCP (μM)	Sulfate(μM)	PCP (μM)	Sulfate	No Sulfate	Sulfate	No Sulfate
0.5	4.20	97	4.00	43	67	67	84
1.0	3.96	136	3.91	34	80	71	99.2
2.0	4.01	375	3.94	38	83	80	99.8
5.0	3.89	731	3.70	48	81	98	99.7
16.7	3.88	2604	3.69	59	82	99.8	100

Observed Transformation Pathway

Transformation of PCP observed in the presence of sulfate at electron demand conditions of 0.5, 1.0, 2.0, 5.0 and 16.7 is summarized in Figure 5.6, Figure 5.7, Figure 5.8, Figure 5.9 and Figure 5.10. Under the sulfate and supplemental electron donor conditions evaluated, the observed pathways of PCP transformations were identical. The reductive dechlorination of PCP was initially catalyzed through the reduction of a chlorine in the *ortho* position resulting in the production of 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP). Almost immediately after the observation of 2,3,4,5-TeCP in the serum bottles the appearance of 3,4,5-trichlorophenol (3,4,5-TCP) was noted. Production of 3,4,5-TCP resulted from the *ortho* dechlorination of 2,3,4,5-TeCP.

The extent of PCP transformation in the presence of low sulfate concentration is shown in Figure 5.6. PCP removal from an initial concentration of 4.17μM was noted through the immediate production of 2,3,4,5-TeCP at hour 14. The following sample at hour 38 marked the appearance of 3,4,5-TCP. With increasing time, concentrations of 2,3,4,5-TeCP and 3,4,5-TCP increased until hour 231 when a maximum concentration of 0.76 μM 2,3,4,5-TeCP was reached. Samples greater than hour 231 showed a slight decrease in 2,3,4,5-TeCP concentrations while, 3,4,5-TCP concentrations continued to increase. Incomplete PCP transformation occurred and at experiment termination PCP, 2,3,4,5-TeCP and 3,4,5-TCP concentrations represented 33%, 13% and 52% of the initial PCP concentration measured in the study. Over the duration of the experiment, the average chlorophenol mass observed was 4.01μM, which correlated well to the initial experimental PCP concentration of 4.17 μM. PCP removal in bottle systems amended with sulfate at 1.0 times the available electron demand showed very similar performance (Figure 5.7). Although slightly higher PCP transformations were observed in serum bottles supplied 2.0 times the

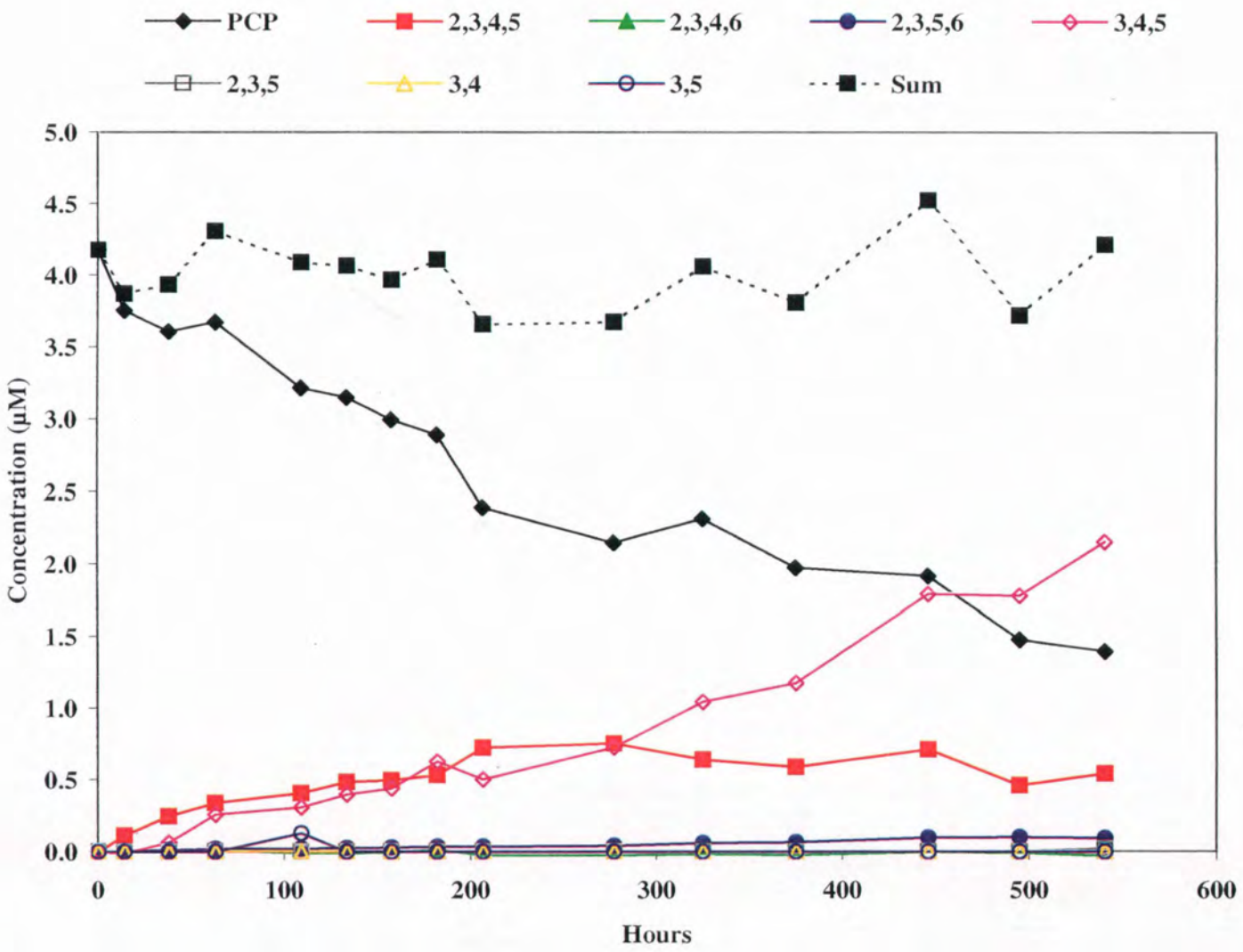

Figure 5.6 PCP degradation pathway observed with sulfate at 0.5x supplied educing equivalents

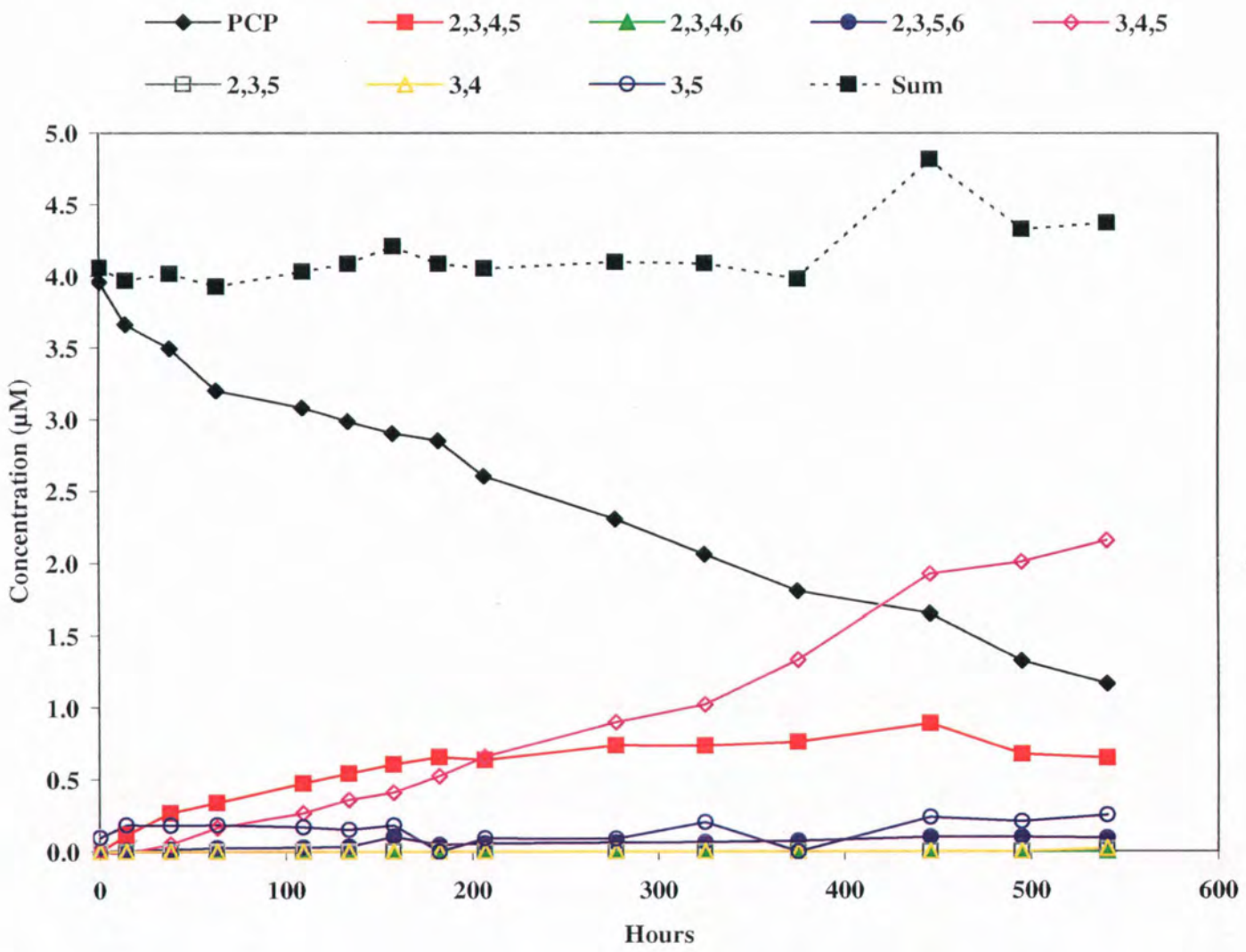

Figure 5.7 PCP degradation pathway observed with sulfate at 1x supplied educing equivalents

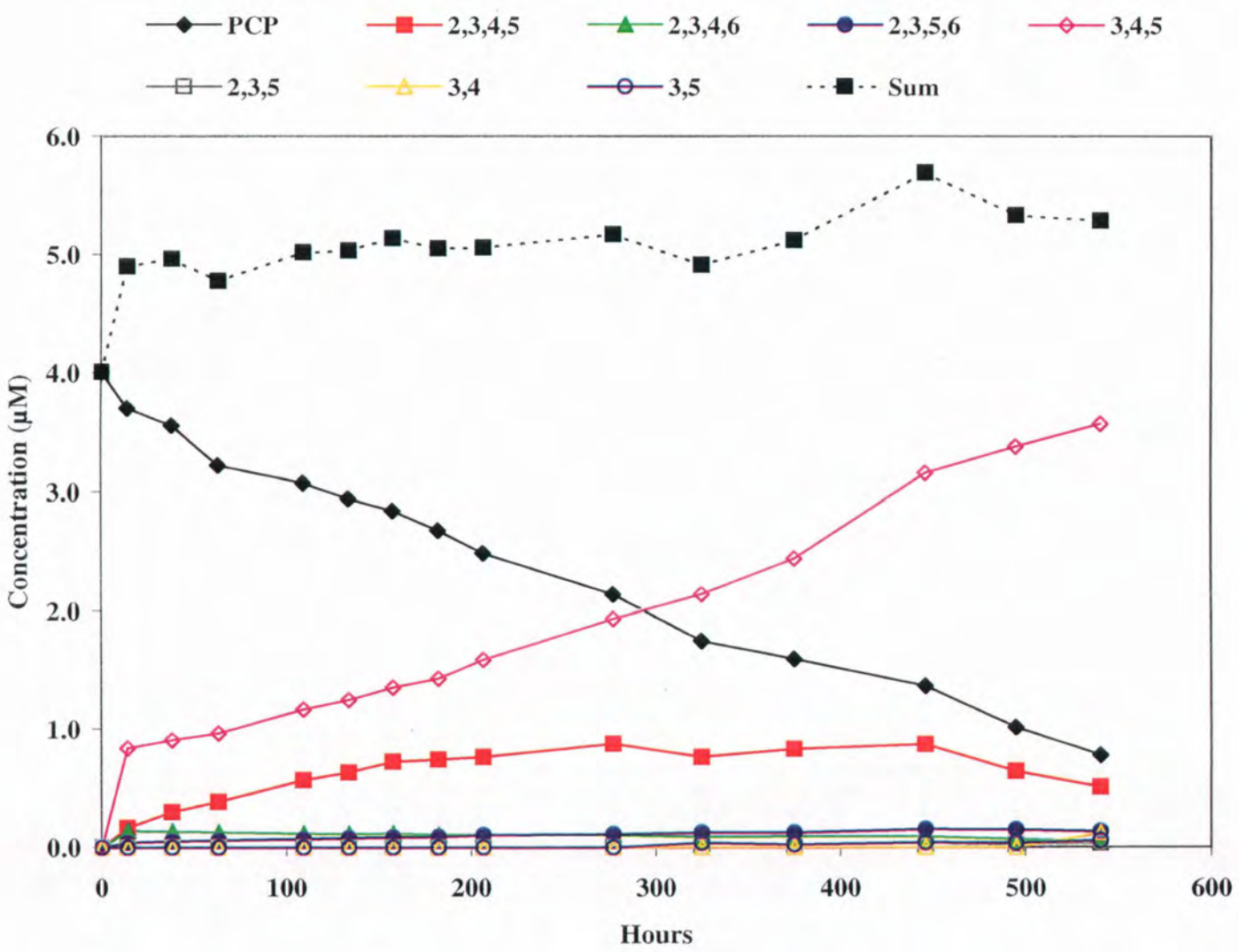

Figure 5.8 PCP degradation pathway observed with sulfate at 2x supplied educing equivalents

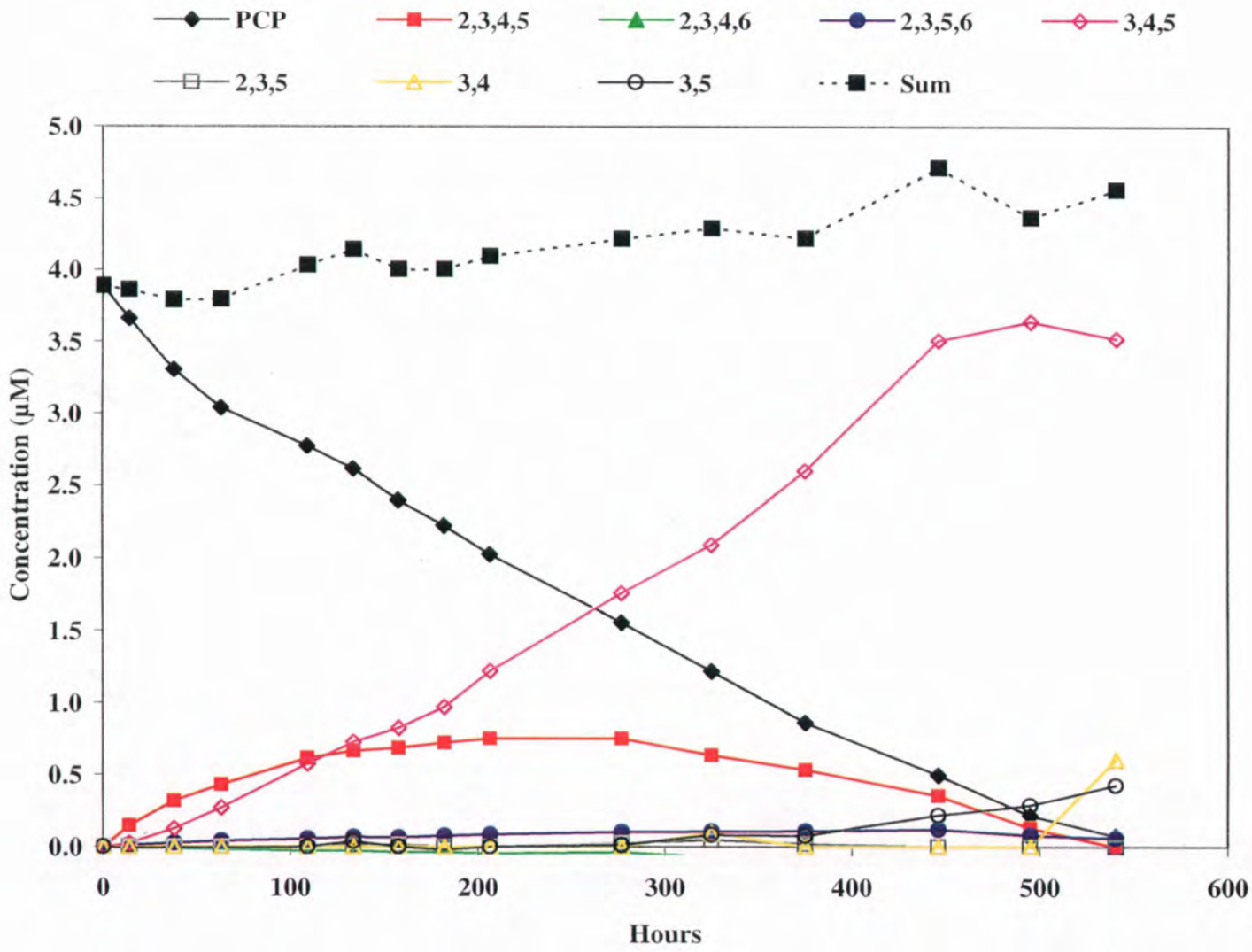

Figure 5.9 PCP degradation pathway observed with sulfate at 5x supplied educing equivalents

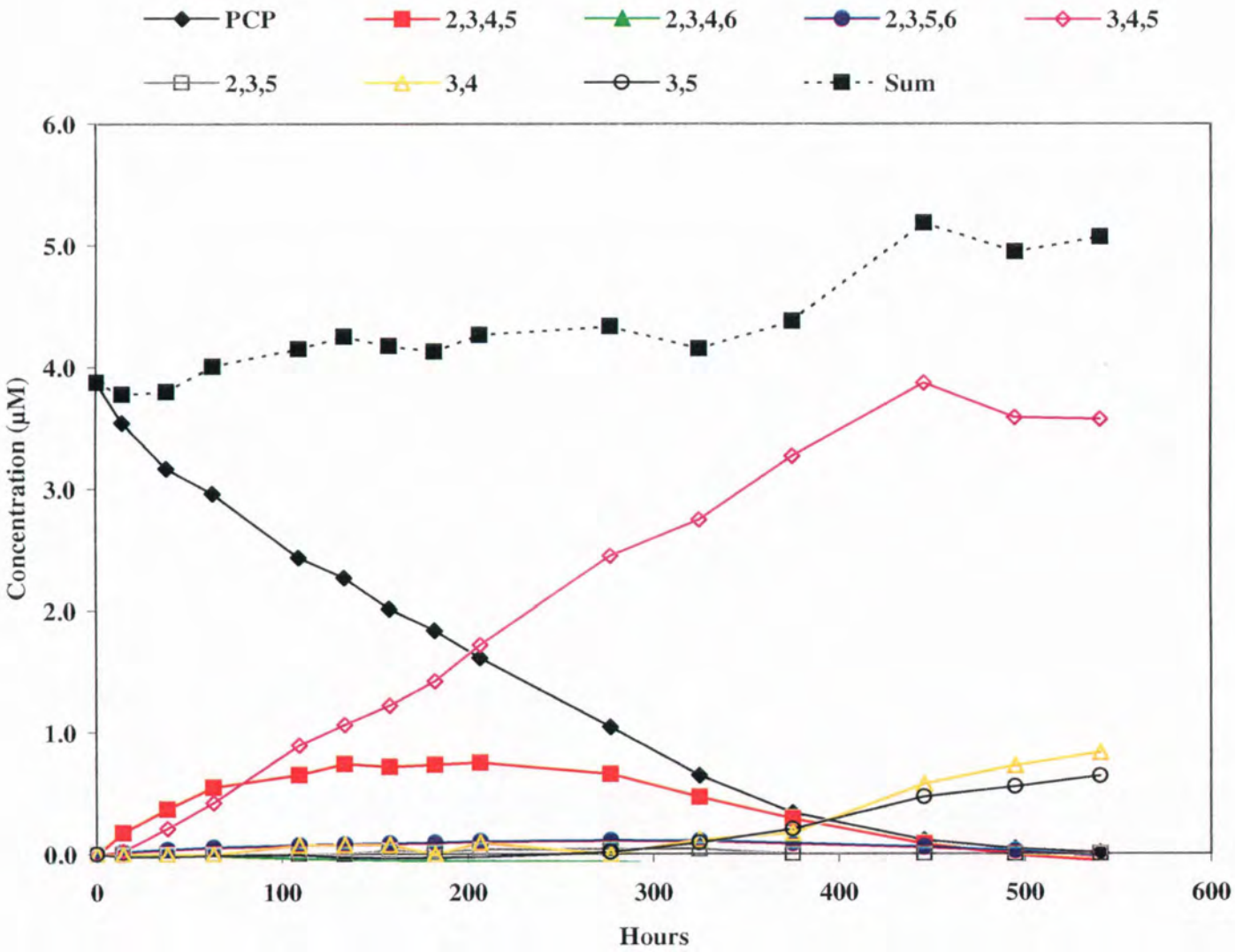

Figure 5.10 PCP degradation pathway observed with sulfate at 16.7x supplied educing equivalents

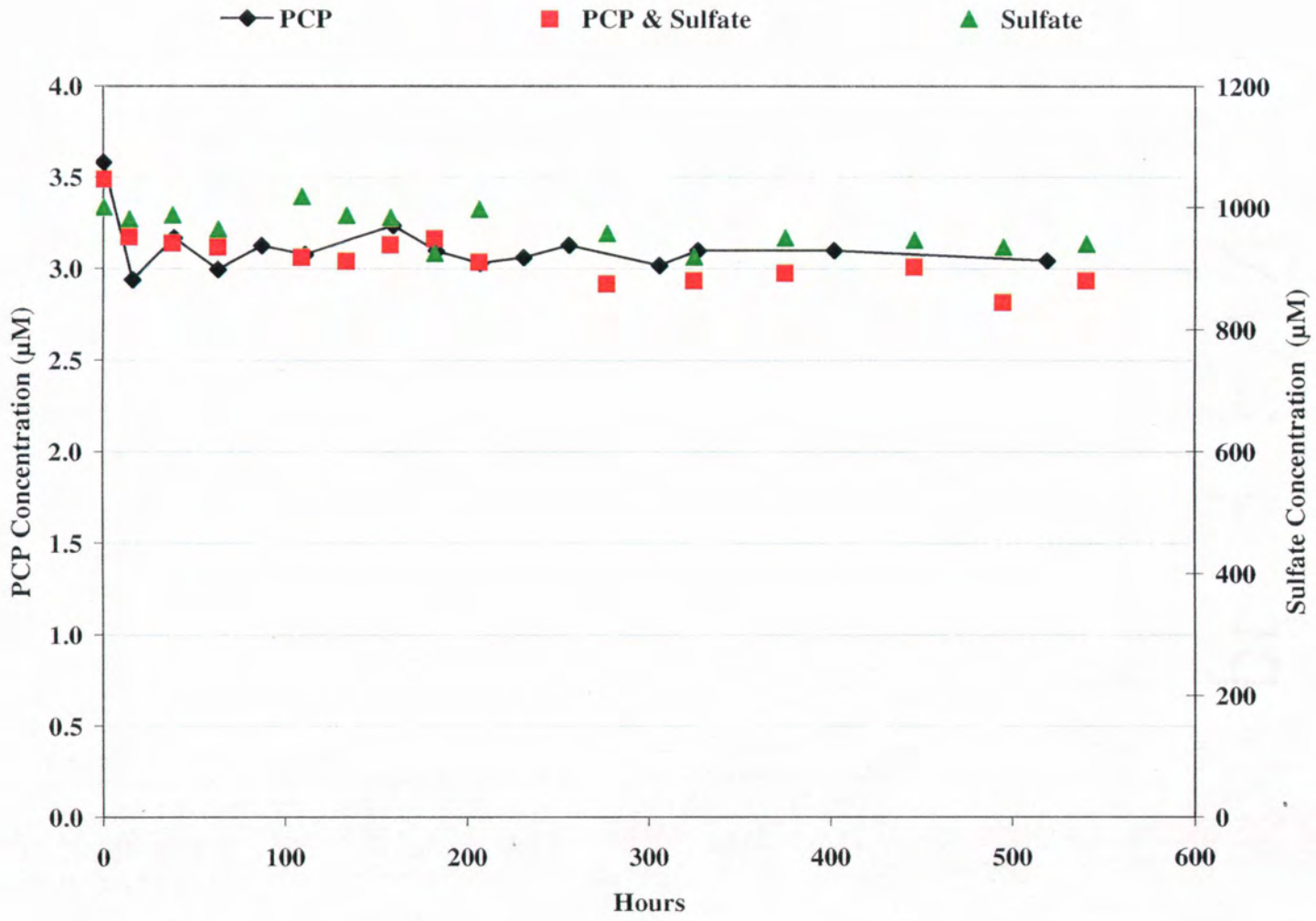

Figure 5.11 The effect of sulfate on PCP degradation in parallel experimental controls

available electron demand, removal of PCP was incomplete (Figure 5.8). Like the previous electron conditions evaluated in Figure 5.6 and Figure 5.7, minimal removal of PCPs ortho dechlorination product 2,3,4,5-TeCP was observed (Figure 5.8).

Under the middle sulfate condition evaluated (Figure 5.9), near complete removal of PCP occurred. PCP was degraded from an initial concentration of 3.9 μM to 0.22 μM over the 540 hour study. PCP degradation gave way to increasing 2,3,4,5-TeCP concentrations, which reached a maximum concentration of 0.76 μM at 207 hours. A small plateau in 2,3,4,5-TeCP concentration was observed between 207 and277 hours. Steady removal of 2,3,4,5-TeCP resumed after hour 325 and continued for the duration of the study. Near complete transformation of 2,3,4,5-TeCP was observed. However, trace quantities (0.002 μM) of 2,3,4,5-TeCP were detected at study termination. At 14 hours, chlorophenol analysis confirmed the presence of 3,4,5-TCP. With increasing time, concentrations of 3,4,5-TCP climbed steadily and accumulated. A maximum concentration of 3.6 μM 3,4,5-TCP was observed at hour 495. In the midrange sulfate bottle, degradation through 3,4,5-TCP was observed through the production of 3,5-dichlorophenol (3,5-DCP) at 277 hours and 3,4-dichlorophenol at 540 hours. Increasing concentrations of 3,5-DCP were noted for times greater than 277 hours. The sum of chlorophenols observed in the experimental system is plotted with time. The average chlorophenol concentration over the experiment, 4.13 μM was slightly higher than the initial PCP (3.89 μM) present in the serum bottle.

At the highest sulfate concentration evaluated Figure 5.10, PCP removal was nearly complete. From an initial concentration of 3.9 μM to 0.009 μM at 540 hours, PCP values continually decreased with increasing time. PCP transformation gave way to immediate production of 2,3,4,5-TeCP and 3,4,5-TCP, which were observed concurrently at hour 14. Transient production of 2,3,4,5-TeCP was noted; 0.76 μM at hour 207 marked the maximum observed concentration. Steady production of 3,4,5-TCP was observed over the course of the experiment. At 446 hours a maximum concentration of 3.9 μM 3,4,5-TCP was observed. Subsequent sampling periods showed a slight decrease in 3,4,5-TCP concentrations. At study termination, 3,4,5-TCP concentrations accounted nearly 100% of the initial PCP concentration measured. After 325 hours, noticeable increases in the concentrations of both 3,4-DCP and 3,5-DCP were observed. Study termination at 540 hours resulted in maximum concentrations of 0.84 μM and 0.64 μM, respectively for 3,4-DCP and 3,5-DCP. The average sum of chlorophenols observed over the course of the experiment, 4.3 μM was slightly higher than the initial PCP concentration of 3.88 μM measured at time zero.

Evidence of Pentachlorophenol Biotransformation

Chlorophenol and sulfate concentrations present in the sterile system as a function of time are shown in Figure 5.11. Over the first sampling interval, a decrease in PCP concentrations was observed in the sterile bottles in the presence or absence of sulfate. PCP concentrations observed after 14 hour remained constant.

Average PCP values in the sulfate free system measured 3.08 ± 0.07 μM while, sulfate amended systems measured 3.03 ± 0.10 μM. Sulfate concentrations observed in the sterile control were very stable over the duration of the experiment and measurements averaged 964±28 μM. The pathway of PCP degradation in the sterile control system is presented in Figure 5.12. Outside of the behavior during the first 14 hours of study, there is no appreciable decrease in PCP concentrations with increasing time. Small concentrations of 2,3,4,6 tetrachlorophenol and 3,4,5-TCP, present in the inoculum, were observed in the control system. The average 2,3,4,6-TeCP and 3,4,5-TCP concentrations (0.14±0.01 μM and 0.12± 0.01 μM, respectively) remained virtually unchanged over the duration of the experiment. The stability of PCP and residual chlorophenols indicates that chlorophenol removal by abiotic processes in the sterile control was negligible. Based upon the observation of stable chlorophenol and sulfate concentrations, there was no evidence to suggest biological activity in the sterile system.

RESULTS: FIELD

During the pilot demonstration at the L.D. McFarland facility, PCP degradation was evaluated in the presence of sulfate, a competitive electron donor. Imitation vanilla served as the electron donor in the permeable barrier experiments. Supplemental COD supply to the treatment unit was constant at 10 mg/L during the competitive acceptor study. Environmental conditions of the reactor system expressed by the apparent redox potential E_H and pH are presented with time in Figure 5.13. The apparent E_H, is referenced to the standard hydrogen electrode and is presented in millivolts (mV). PCP measurements in the permeable barrier reactor are based upon the analysis of 28 independent samples collected within the reactor assembly. Average PCP influent concentration was estimated from nine measurements of PCP observed in a monitoring well approximately 3 feet upgradient. Vertical partitions in Figure 5.13 and Figure 5.14 denote the experimental conditions present in the permeable barrier reactor.

The Effect of Sulfate on in-situ Environmental Conditions

The effect of sulfate addition on the environmental conditions present in the reactor system is clearly shown in Figure 5.13. Prior to sulfate injection on June 1, redox measurements within the reactor were stable and hovered around –300 mV in both treatment zones. Following the addition of sulfate to the system, a steady increase in system apparent E_H was observed. Conditions in treatment zone A and B were nearly identical as apparent E_H measurements increased. Maximum values were reached almost immediately and measured 0 and 30 mV in treatment zone A and B, respectively. During the period of sulfate injection a slow decrease in apparent E_H measurements was observed in treatment zone A. Measurements from zone B showed no appreciable change during this period. When sulfate supply to the reactor system was terminated, apparent E_H measurements in treatment zone A fell rapidly, while zone B measurements remained stable. Apparent E_H measurements within treatment zone A decreased steadily from zero mV to approximately –180 mV before stable environmental conditions were observed. In the

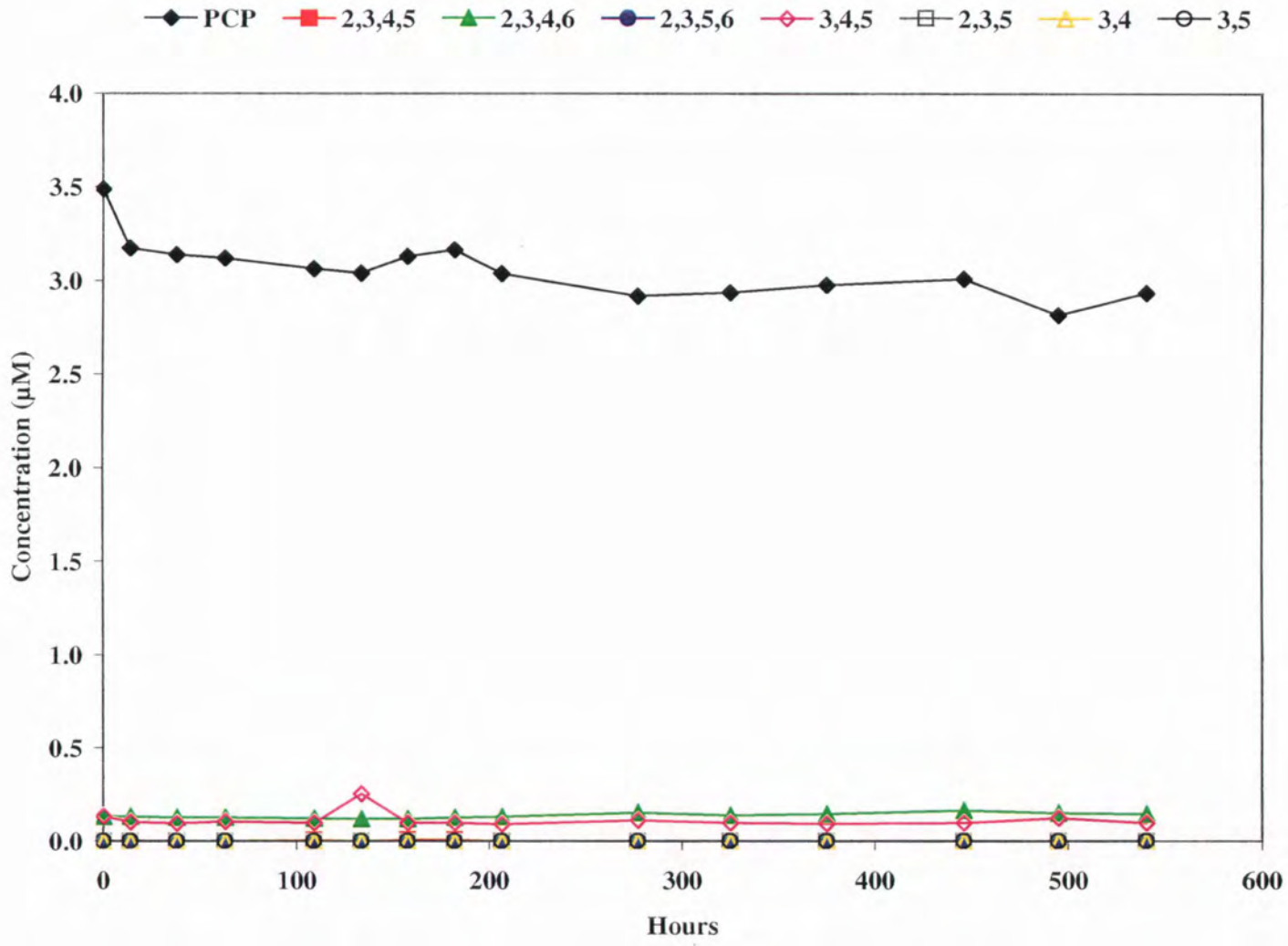

Figure 5.12 PCP degradation pathway observed in parallel experimental controls

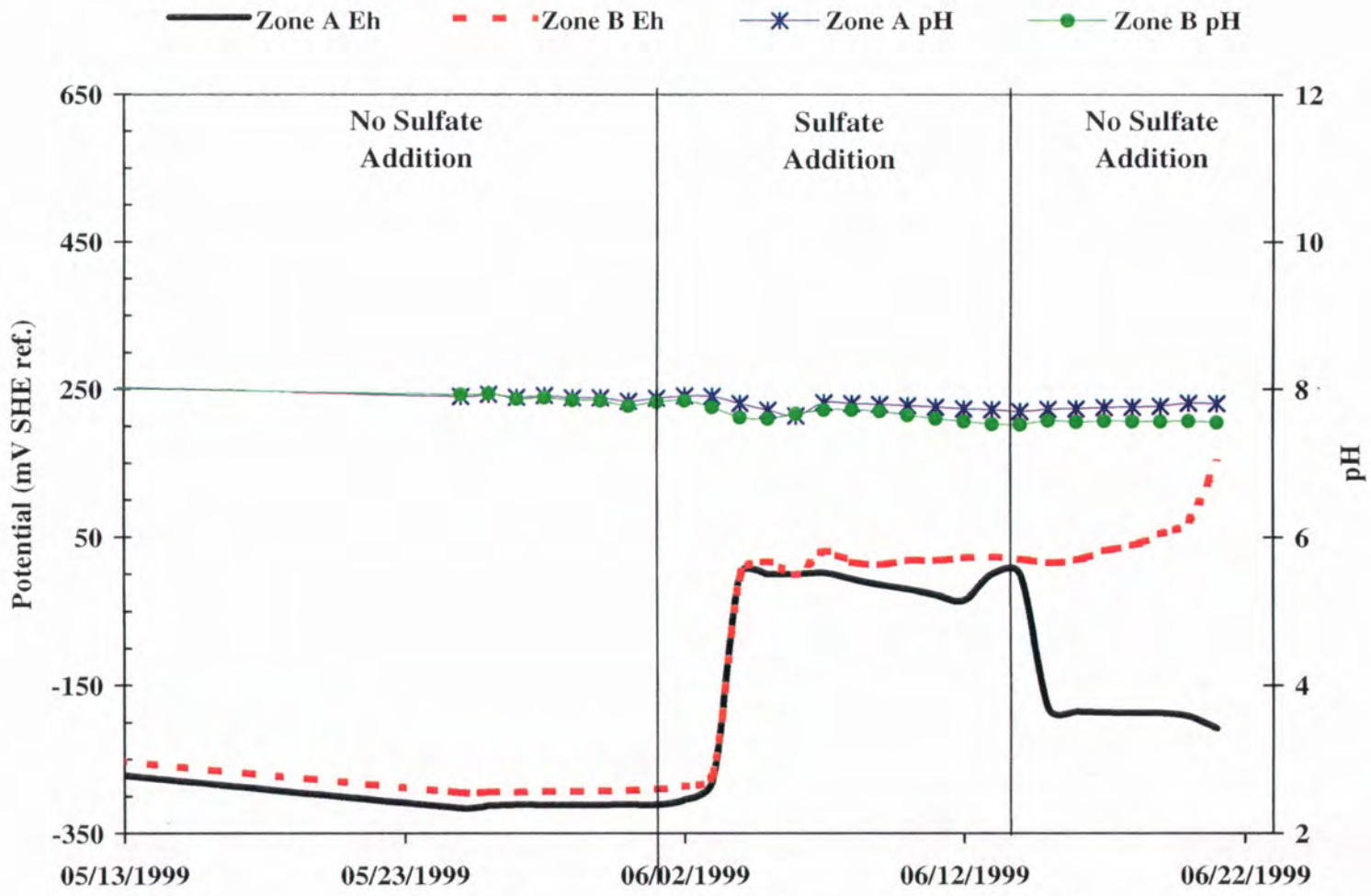

Figure 5.13 Reactor environmental conditions as a function of sulfate addition

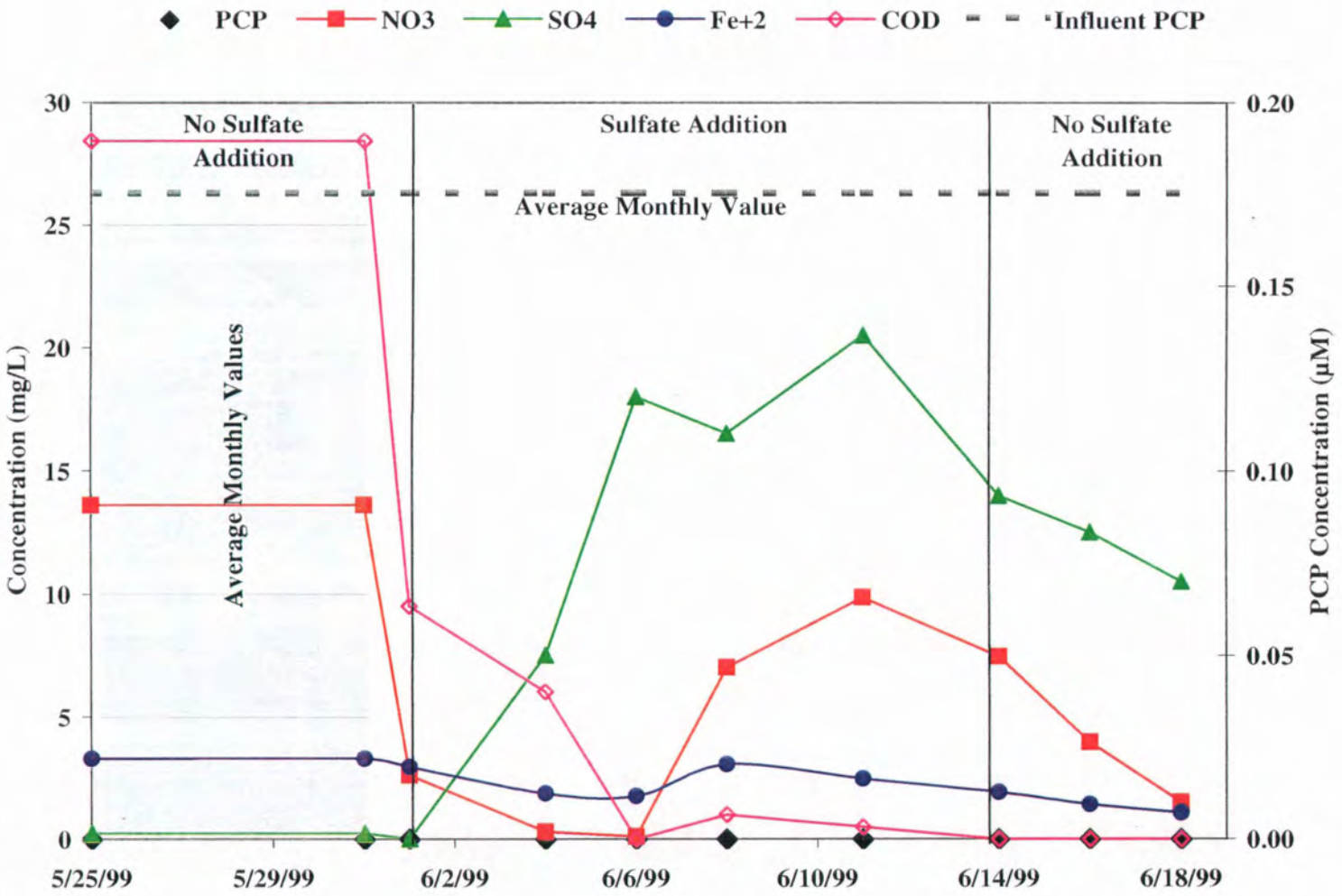

Figure 5.14 The effect of alternate electron acceptors on in-situ PCP degradation

absence of sulfate supply, apparent E_H measurements collected from zone B showed increasing potential with increasing time. Treatment zone A and B pH over the duration of the experiment was constant; pH values averaged 7.8±0.1 and 7.7±0.1, respectively.

The Effect of Sulfate on in-situ Pentachlorophenol Degradation

The effect of sulfate on the *in-situ* degradation of PCP is shown in Figure 5.14. To estimate the average PCP influent to the reactor, PCP concentrations upgradient of the treatment system were monitored. An average upgradient value 0.18 μM PCP was observed under the field conditions evaluated. Despite the changing environmental conditions, in the presence or absence of sulfate, *in-situ* PCP degradation was complete. At all observed locations and under all sample sets collected, there was no detectable concentration of PCP in the permeable barrier reactor. In addition, complete removal of metabolic products formed through anaerobic PCP degradation was also observed.

Characterization of groundwater samples from the permeable barrier reactor revealed the presence of nitrate, iron (II) and background COD. Sulfate was not detected in the treatment system prior to injection on June 1. An immediate response to sulfate addition was observed and concentrations climbed steadily to reach a maximum value of 20.5 mg/L. Concurrent to increasing sulfate concentrations was the observed decrease in background COD values. Normally, observed in the range of 20 mg/L, background COD was completely exhausted shortly after sulfate injection commenced. Samples collected on the June 8 showed a transient increase in background COD however, values quickly approached zero in subsequent sampling events. Iron (II) concentrations in the reactor system also decreased during the period of sulfate injection. Nitrate concentrations fluctuated over the experimental period. Decreasing nitrate concentrations were observed with increasing time until a transient spike occurred on June 6. Nitrate concentration climbed briefly and reached a maximum value of 10 mg/L before removal was observed.

DISCUSSION

The effect of sulfate on the reductive dechlorination of a PCP was studied in laboratory serum bottles. While the addition of sulfate to the serum bottles was not inhibitory to reductive dechlorination, a marked effect was present in the overall rates of PCP transformation. In comparison to PCP degradation in the absence of sulfate, systems amended with sulfate generally showed slower removal rates (Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.4 and Figure 5.5). In the absence of sulfate, PCP removal appears to proceed in a first order fashion. Degradation in the presence of sulfate progresses in a linear fashion, suggestive of zero order reaction kinetics. In studies conducted with estuarine sediments, naturally high in sulfate reducing bacteria, equivalent PCP transformation rates were observed in the presence or absence of sulfate addition (Liu et al., 1996). Our studies however, show a distinct difference in PCP transformation rates relative to the presence of sulfate. The lack of sulfate removal in the serum bottles also suggests that

the culture harvested from the permeable barrier reactor contained a low population of sulfate reducing bacteria.

The Effect of Sulfate of Pentachlorophenol Degradation

In the serum bottles studied, PCP transformation occurred in the presence and absence of sulfate. Although PCP transformation rates differed, the extent of PCP transformation was generally much greater than sulfate. These results seem to indicate that sulfate-reducing bacteria were not responsible for the observed transformation of PCP. While experimental results fail to support the coupling of reductive dechlorination and sulfate reduction, several groups have shown the two processes are closely related. Through the addition of sodium molybdate, a specific inhibitor of sulfate reduction, two studies using estuarine sediments have shown the inhibition of both reductive dechlorination and sulfate reduction (Häggblom and Young, 1990; Liu et al., 1996). The findings suggest that reductive dechlorination is also coupled to sulfate reduction. Studies in the absence of specific inhibitors have also shown a couple between reductive dechlorination and sulfate reduction (Häggblom et al., 1993b; Masunaga et al., 1996).

The performance of the individual sulfate treatments was somewhat surprising. In general, serum bottles, which contained the lowest sulfate concentration, exhibited the slowest removal of PCP. Degradation of PCP as a function of sulfate concentration is shown in Figure 5.15. Initial PCP concentration in all the serum bottles evaluated measured roughly 4 μM. Steady degradation was observed with increasing time for all sulfate concentration examined with the exception of the poisoned control system. The greatest rate of sulfate removal was observed in serum bottles where sulfate was supplied at 16.7 times the available electrons released from the oxidation of the initial donor concentration used. Transformation rates decreased with decreasing sulfate concentrations. Results seem to indicate that increasing sulfate concentrations enhanced PCP degradation. In soil systems, sulfate addition was shown to increase the rate of PCP reductive dechlorination (Chang et al., 1996).

It is possible that the trends observed in PCP removal rates are a function of the experimental conditions. Competitive acceptor studies were conducted in serum bottles previously used to evaluate the effect of electron donor concentration. Evaluation of Figure 5.15 in light of the previous experiment conducted in the serum bottles shows that PCP degradation is the fastest in bottles that were previously evaluated with the highest substrate concentration. Conversely, the slowest PCP removal rates were observed in bottles that previously contained the lowest substrate concentration (Chapter 4). Judging from the independence of PCP transformation rates on electron donor concentration it is unlikely that the substrate concentrations alone were responsible for the performance trends observed in the sulfate amended systems. Growth of microbial mass at higher substrate concentrations however, cannot be ruled out. While an evaluation of solids was not conducted during this study, it is very possible that a disproportionate mass of organisms among the various sulfate concentrations investigated was responsible for the observed difference in PCP transformation rates.

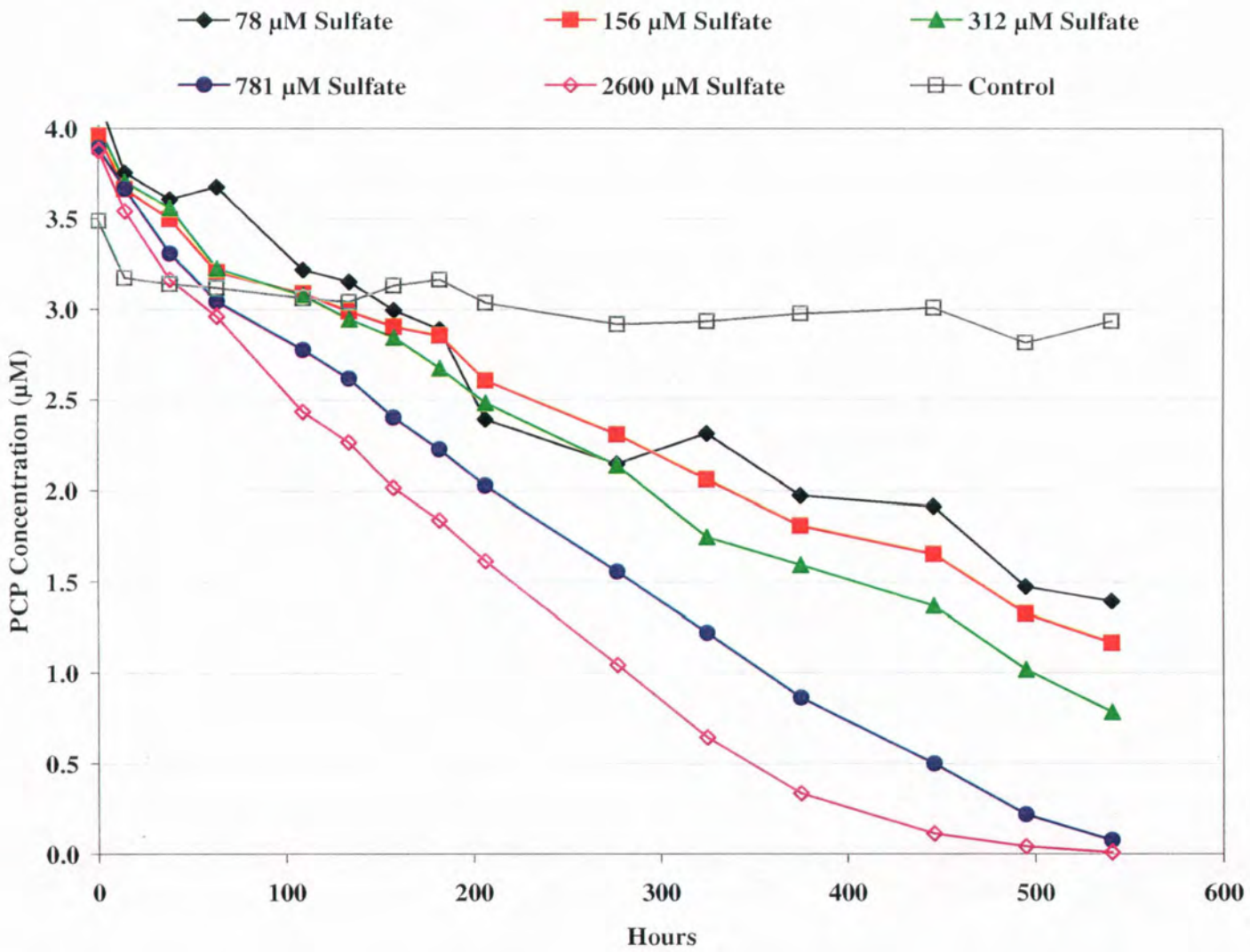

Figure 5.15 The effect of sulfate concentration on PCP degradation

Degradation Pathway Observed

Experimental observations indicate that while sulfate effected the rate of PCP transformation it had no effect on the degradation pathway. In Figure 5.6, Figure 5.7, Figure 5.8, Figure 5.9 and Figure 5.10, the reductive dechlorination pathway of PCP is identical. Degradation proceeded through the sequential removal of PCPs *ortho* chlorine resulting in 3,4,5-TCP. The accumulation of 3,4,5-TCP in the system was observed in all active serum bottles. However, further removal to 3,4-DCP and 3,5-DCP is evident. It is unlikely the incomplete transformation of PCP and the accumulation of 3,4,5-TCP can be attributed to the presence of sulfate. Despite differences in sulfate concentrations, the extent of PCP degradation in the systems evaluated was nearly identical. The concentration of 3,4,5-TCP in the system may have been inhibitory to further degradation. However, anaerobic degradation of 3,4,5-TCP in the presence of sulfate has been reported (Masunaga et al., 1996). Furthermore, previous studies conducted in the absence of sulfate also showed that PCP was exclusively degraded to 3,4,5-TCP, which accumulated (Cole and Woods, 2000c).

Evaluation of PCP concentrations in the experimental controls (Figure 5.11) clearly shows that abiotic mechanisms of removal were negligent. Sulfate concentrations remained stable with time and there was no evidence to suggest biological reduction. Comparison of active chlorophenol degradation in Figure 5.6, Figure 5.7, Figure 5.8, Figure 5.9 and Figure 5.10 to the performance of the poisoned control (Figure 5.12) clearly indicate that the transformation of PCP in the active serum bottles was biologically meditated. Biological transformation in the active systems is supported by the transient production and consumption of reaction products, which originated from the reductive dechlorination of PCP. The absence of these metabolic degradation products is evident in the poisoned control system. Abiotic removal of chlorophenols in the active system was minimal. Chlorophenol mass balances tabulated over the duration of the experiment accurately represent the initial molar mass of PCP present in the serum bottles (Figure 5.6, Figure 5.7, Figure 5.8, Figure 5.9 and Figure 5.10). In general, mass balances plotted in continue to increase with increasing time. The production of 3,4-DCP and 3,5-DCP from 3,4,5-TCP initially present in the system is responsible for stoichiometric conversion of chlorophenols in excess of the initial PCP concentration.

In-situ Pentachlorophenol Degradation in the Presence of Sulfate

In the field, the addition of sulfate to the permeable barrier reactor was evident in changes to the system environmental conditions (Figure 5.13). The apparent E_H in the reactor system followed the supply of sulfate and resulted in a steady state apparent E_H around +40 mV. During this period, PCP degradation was not affected (Figure 5.14). PCP measurements at all time and space in the treatment system were zero. The elevated apparent E_H conditions in the reactor apparently had no effect on PCP degradation. Comparison of standard potentials Table 5.1 and Table 5.2, show that chlorophenol reduction is possible under environmental conditions where nitrate reduction is favored (ca, +400 mV) which is significantly

higher than conditions observed during sulfate injection. However, while thermodynamically feasible, most chlorophenol transformations are observed in systems that are highly reducing (ca, –270 mV) and typical of sulfate-reducing or methanogenic environments. Continued PCP degradation under relatively high apparent E_H conditions was an interesting observation. While degradation was unaffected over the short term, operation of treatment system under the apparent E_H conditions brought on by sulfate injection would likely result in a decrease in the efficiency of PCP transformation.

While appreciable sulfate removal was not observed under laboratory conditions, significant reduction of sulfate was suspected in the field experiments. Sulfate was supplied to the permeable barrier reactor to provide approximately 1000 μM (100 mg/L), which was chosen to minimize the potential for sulfide toxicity to the treatment system. Concentrations of sulfide as low as 3 mM were shown inhibitory to methanogenesis and sufidogenesis by several research groups (Maillacheruvu et al., 1993; Uberoi and Bhattacharya, 1995). Complete reduction of the sulfate supplied in the field would have resulted in sulfide concentration 1/3 of levels reported as inhibitory. Partial sulfate reduction was observed in the permeable barrier reactor. The maximum sustained concentration measured over the period of sulfate injection was about 200μM (20 mg/L). Prior to sulfate injection, background COD in the reactor system averaged around 30 mg/L. Shortly after sulfate was introduced, background COD measurements fell rapidly and approached zero. It is likely that the observed decrease in COD was a result of a microbial redox couple that reduced exogenous sulfate at the expense of the background organic loading.

Sulfide measurements were not conducted during the period of sulfate injection. The presence of iron (II) in the system would have likely interfered with sulfide measurement in the field. On occasion, the characteristic odor of hydrogen sulfide was noticed in the process trailer but no formal analysis program for dissolved gases in the permeable barrier reactor was undertaken. Iron(II) in the presence of sulfide forms insoluble complex and will precipitate from solution. The addition of excess iron (II) to biological systems reducing sulfate has been successfully used to attenuate sulfide toxicity in laboratory experiments in batch and continuous stir reactor (Gupta et al., 1994a; Gupta et al., 1994b; Gupta et al., 1996a).

Evaluation of iron (II) measurements in the field during the period of sulfate injection supports precipitation of insoluble iron sulfide complexes (Figure 5.14). A noticeable decrease in soluble iron (II) concentrations follows the corresponding increase in sulfate concentrations measured in the permeable barrier reactor system. A dense black floc in process sample lines and within the flow cells used to monitor environmental conditions lends further support to soluble sulfide removal through iron precipitation. The behavior of nitrate in the reactor system during sulfate injection was surprising. Perhaps electron flow normally used for reduction of background nitrate was interrupted by the presence of sulfate. Environmental conditions may have favored sulfate reduction, which temporally channeled electrons away from nitrate reduction resulting in the observed concentration increase.

SUMMARY AND CONCLUSIONS

Anaerobic processes are an effective mechanism for the biological treatment of highly chlorinated organic compounds. In the deployment of an anaerobic strategy for *in-situ* groundwater treatment, interference from competing biological processes exists. To evaluate the effect of competing biological processes, PCP reductive dechlorination was evaluated in the presence of sulfate, under laboratory and field conditions. Laboratory experiments revealed that sulfate was not inhibitory to PCP reductive dechlorination. However, laboratory PCP transformation rates in the presence of sulfate were noticeably slower than comparable systems, which lacked sulfate. Sulfate addition to the pilot scale reactor had no apparent effect on the extent of PCP degradation. Complete PCP removal occurred in the system regardless of sulfate addition. The laboratory degradation pathway of PCP in the presence or absence of sulfate was identical. Laboratory degradation of PCP proceeded by reductive dechlorination and followed sequential *ortho* chlorine cleavages to yield 3,4,5-TCP. In the serum bottles evaluated, 3,4,5-TCP accumulated. In the field, PCP was fully degraded; no metabolic products of reductive dechlorination were observed. Overall, results of this study suggest that anaerobic processes could be successfully implemented for the biological remediation of groundwater impacted by sulfate and chloroaromatic compounds. Comparable behavior in system operation between field and laboratory experiments illustrates the utility of companion studies for the optimization of *in-situ* remediation systems.

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CHAPTER 6

THE EFFECTS OF HYDROGEN ON THE REDUCTIVE DECHLORINATION OF 3,4,5-TRICHLOROPHENOL

Jason David Cole

ABSTRACT

In computer monitored batch reactor system, the reductive dechlorination of 3,4,5-trichlorophenol (3,4,5-TCP) was evaluated as a function of hydrogen partial pressure. Hydrogen gas supplied to the reactor was controlled though computer operated mass flow controllers. The configuration of the gas supply system allowed for the precise control of gas flow rate and composition. At a hydrogen partial pressure of 0.12 atm in the reactor headspace, the reductive dechlorination of 3,4,5-TCP was observed. Removal of 3,4,5-TCP from the reactor corresponded with the exclusive production of 3,5-dichlorophenol. No other metabolic products of 3,4,5-TCP reductive dechlorination were detected. Production of 3,5-DCP was not stoichiometric with 3,4,5-TCP concentrations, which suggests degradation of 3,5-DCP was also occurring. Step reductions in the reactor hydrogen partial pressure were made in an effort to determine the minimum hydrogen concentration required for 3,4,5-TCP degradation. Hydrogen partial pressures evaluated between 0.12 atm and 0.00002 atm, which corresponded to an aqueous concentration range of 89μM to 15 nM effectively, supported 3,4,5-TCP reductive dechlorination. The reductive dechlorination of 3,4,5-TCP ceased when the supply of hydrogen to the reactor was terminated. In 125 hours of operation, 3,4,5-TCP was removed from an initial concentration of 1.6 μM to 0.27 μM. The aqueous concentration of 3,4,5-TCP was increased in the system; in the absence of hydrogen addition, 3,4,5-TCP was not removed. Degradation of 3,4,5-TCP resumed following the addition of hydrogen to the reactor headspace. After a short period of active 3,4,5-TCP removal, degradation unexpectedly ceased. Despite hydrogen partial pressures previously shown amendable to 3,4,5-TCP degradation, continued hydrogen addition failed to stimulate reductive dechlorination. After a lag of nearly 120 hours, 3,4,5-TCP reductive dechlorination resumed at a hydrogen partial pressure of 0.03 atm. With increasing time, 3,4,5-TCP was steadily removed from the system. While the complete removal of 3,4,5-TCP was not observed, transformations following each concentration spike measured 82% and 85%, respectively.

RESEARCH OBJECTIVES

In anaerobic pentachlorophenol (PCP) degrading serum bottles using imitation vanilla flavoring as an electron donor, PCP was degraded by reductive dechlorination. PCP was exclusively degraded through sequential reductions at *ortho* carbon atoms producing 3,4,5-TCP. With time, 3,4,5-TCP accumulated in all serum bottles. Although 3,4,5-TCP removal was observed, it was considerably slower than the rate of production from PCP reductive dechlorination (Cole and Woods, 2000c; Cole and Woods, 2000d). It was hypothesized that the serum bottles were hydrogen limited which, resulted in the accumulation of 3,4,5-TCP in the system. In an effort to determine the relationship between 3,4,5-TCP reductive dechlorination and hydrogen partial pressure, laboratory experiments were conducted with the following objectives:

- To evaluate the potential of 3,4,5-TCP reductive dechlorination when hydrogen is supplied as an exogenous electron donor.
- To estimate the threshold hydrogen concentration requisite for 3,4,5-TCP reductive dechlorination.
- To investigate the pathway of 3,4,5-TCP degradation when hydrogen serves as the electron donor.
- To examine the potential for the use of hydrogen in a field based remediation scheme.

INTRODUCTION

Microbial degradation of pentachlorophenol (PCP) has been studied using pure cultures and microbial consortiums. The mechanisms of degradation are well understood and have been evaluated under both aerobic and anaerobic conditions (Häggblom, 1990; Häggblom, 1992; Mohn and Tiedje, 1992). Under anaerobic conditions, PCP reductive dechlorination may result in wide array of metabolic products. As such, the pathway of PCP degradation may have a profound effect on the overall success of microbial-based remediation scheme. Production and accumulation of intermediate metabolites, especially those more toxic than the parent compounds, is a reoccurring problem in the application of biologically based treatment regimes (Zitomer and Speece, 1993). Therefore, successful application of biological treatment techniques requires a thorough understanding of the target compound's degradation pathway.

PCP reductive dechlorination may occur at any of the chlorine substituted positions (Mikesell and Boyd, 1986; Mikesell and Boyd, 1988; Nicholson et al., 1992). However, relative to consortium acclimation, trends in the position of initial chlorine removal exist. In general, consortia not acclimated to chlorophenols preferentially remove chlorines in the *ortho* position (Boyd and Shelton, 1984; Boyd et al., 1983; Mikesell and Boyd, 1985; Woods, 1985). Chlorophenol acclimated organisms are reported to remove chlorines from all positions on the aromatic ring. However, regardless of acclimation state, removal of PCP's *ortho* chlorine atoms is favored over the *meta* and *para* substituted positions (Mikesell and Boyd, 1985; Mikesell and Boyd, 1986; Mikesell and Boyd, 1988; Woods, 1985). It is unclear what factors are responsible for the predominance of PCP degradation by *ortho* chlorine removal.

Reductive dechlorination of PCP by sequential removal of *ortho* substituted chlorine atoms results in the production of 3,4,5-trichlorophenol (3,4,5-TCP). Many groups have reported the complete degradation of PCP through a sequential *ortho* reductive dechlorination pathway without interference from 3,4,5-TCP (Chang et al., 1998a; Larsen et al., 1991; Liu et al., 1996; Madsen and Aamand, 1991; Mikesell and Boyd, 1986). In PCP degrading anaerobic systems lacking exogenous substrates, 3,4,5-TCP degradation was observed however, PCP and 2,4,6-trichlorophenol degradation were inhibited (Krumme and Boyd, 1988). Conversely, accumulation of 3,4,5-TCP from PCP reductive dechlorination has also been observed (Cole et al., 1996; Hendriksen and Ahring, 1993; Madsen and Licht, 1992; Stuart and Woods, 1998). Transient

accumulation of 3,4,5-TCP resulting from the inequality in production and removal rates has also been suggested as the rate limiting step in PCP reductive dechlorination by a sequential *ortho* dechlorination pathway (Hendriksen and Ahring, 1993; Liu et al., 1996).

Metabolites of PCP reductive dechlorination vary in their degree of microbial toxicity. Evaluation of individual chlorophenols and PCP against various bacterial species indicated in 26 of 30 strains tested, 3,4,5-TCP exhibited the highest toxicity (Ruckdeschel et al., 1987). These findings were supported by Wu et al., 1993 who determined at equal concentrations of 1.0 mg/L, 3,4,5-TCP was more toxic than PCP. Toxicity assays by Mikesell and Boyd, 1986 and Bryant et al., 1991 found 3,4,5-TCP 5 times more mutagenic than PCP. Concentrations of 3,4,5-TCP greater than 5 mg/l were shown to inhibit methanogenesis (Woods, 1985). In addition, fermentative organisms have also shown high sensitivity to 3,4,5-TCP exposure (Madsen and Aamand, 1992). The effects of 3,4,5-TCP production in an anaerobic PCP treatment system are clear. However, the factors that determine the ability of a microbial consortium to tolerate or degrade 3,4,5-TCP remain unknown.

Chlorophenol reductive dechlorination has been shown with a variety of organic electron donors (Beaudet et al., 1998; Chang et al., 1998b; Hendriksen et al., 1992; Larsen et al., 1991). Electrons released in the biological oxidation of the donor are believed to participate directly in the reduction of the chlorophenol (Mohn and Tiedje, 1992). However, there is growing evidence to support the hypothesis that an inorganic electron donor, hydrogen provides electrons for chlorophenol reduction. Hydrogen was shown as an electron donor the reductive dechlorination of 3-chlorobenzoate by an obligate anaerobe *Desulfomonile tiedjeii* (Mohn and Tiedje, 1992). Although hydrogen may serve as the electron donor for the reductive dechlorination of 3-chlorobenzoate by *D.tiedjeii*, it is unclear whether hydrogen plays the same role in the dechlorination reactions mediated by the mixed anaerobic culture. Stuart and Woods recently reported the reductive dechlorination of PCP through endogenous hydrogen production by acetoclastic methanogens (Stuart and Woods, 1999). Studies evaluating the degradation of chlorinated alkenes also show a link between hydrogen and reductive dechlorination (DiStefano et al., 1992; Maymo-Gatell, 1995).

Hydrogen plays an important role in controlling the external conditions present in the anaerobic environment. Whether biologically-derived through fermentation or volatile acid degradation or produced abiotically thorough metal corrosion hydrogen, can support the electron donor requirements for many members of the anaerobic community (Brock and Madigan, 1991; Lorowitz et al., 1992). As such, competition exists among the many species of organisms that have developed mechanisms to use hydrogen as an electron donor. In anaerobic systems where organisms directly compete for hydrogen, those with lowest half velocity coefficients (K_s) are generally the most successful at sequestering the electron donor.

Sulfate reducing bacteria for example, typically possesses K_s values, which are lower than those of methanogens. The lower K_s of the sulfate reducing bacteria provide a distinct advantage in hydrogen

competition over methanogens especially at lower hydrogen concentrations (Kristjansson et al., 1982; Robinson and Tiedje, 1984). Studies of aquifer hydrogen concentrations and geochemical speciation conducted by Lovley et al., 1994 corroborate with the ability of sulfate reducers to thrive at hydrogen levels lower than those required for methanogenesis. Recently, hydrogen Ks values for populations degrading chlorinated solvents by reductive processes have been reported. Work conducted by Smatlak et al., 1996 reported Ks values of 20-100 nM hydrogen for tetrachloroethene (PCE) reductive dechlorination by a mixed anaerobic culture. Ballapragada et al., 1997 reported a hydrogen Ks range of 9-21 nM for PCE degradation. Despite the difference in Ks values observed, when hydrogen Ks for reductive dechlorination is compared to a typical hydrogen Ks for methanogenesis, 1000nM, it is clear that when low hydrogen conditions exist, organisms capable of reductive dechlorination will dominate.

Thermodynamic evaluation of methanogenesis, sulfate reduction and reductive dechlorination indicates that when hydrogen serves as the electron donor, reductive dechlorination yields the greatest change in free energy. Evaluation of the thermodynamic relationships at the point where energy is no longer released defines the threshold value for which a reaction will proceed. Since the hydrogen threshold for sulfate reduction is nearly one order of magnitude lower than methanogenesis, sulfate reducers can out compete methanogens at lower hydrogen levels. Organisms capable of reductive dechlorination therefore would posses hydrogen thresholds lower than both sulfate reducing bacteria and methanogens. Studies conducted by Smatlak et al., 1996 estimated a hydrogen threshold concentration of less than 2 nM for PCE reductive dechlorination. Findings were supported by Yang and McCarty, 1998 who determined a 2 nM hydrogen threshold for a mixed anaerobic culture degrading *cis*-1,2-dichlorethene. Recent work by Loffler et al., 1999 reported hydrogen threshold concentrations for PCE reductive dechlorination as low as 0.12 nM. Interestingly when the chlorinated electron acceptor, PCE was removed from the experimental system, threshold hydrogen concentrations, driven by acetogens, were higher (250 nM). Lower hydrogen thresholds observed in systems amended with chlorinated electron acceptors indicated that reductive dechlorination was the terminal electron accepting processes.

Enhancement of reductive dechlorination with electron donors that degrade slowly to maintain low hydrogen partial pressures has been proposed (Smatlak et al., 1996; Fennell et al., 1997; Yang and McCarty, 1998; Loffler, et al., 1999). Based upon the thermodynamic predictions of threshold hydrogen concentrations and experimental observations presented in the degradation of chlorinated solvents this approach is sound in theory and practice. The addition of hydrogen to chlorophenol degrading systems has produced mixed results. Hydrogen addition was shown to inhibit PCP reductive dechlorination by a methanogenic consortium (Juteau et al., 1995a). Perkins et al., 1994 examined the role of hydrogen, acetate, and fructose as donors for the reductive dechlorination of 2,4,6-trichlorophenol. Under all electron donors evaluated, pathways of reductive dechlorination were identical. Yet, hydrogen spiked bottles exhibited the slowest rates of 2,4,6-trichlorophenol removal.

Based upon the threshold hydrogen concentrations and the physiological differences among microbial groups, the apparent lack of chlorophenol degradation may have resulted from hydrogen concentrations that were too high which, allowed for competing processes e.g., methanogenesis or acetogenesis to occur. Conversely, in freshwater lake sediments, (Zhang and Wiegel, 1990) found that the dechlorination of 2,4-dichlorophenol could be stimulated by the addition of hydrogen to the gas phase. Depletion in endogenous hydrogen concentrations has also shown to slow PCP transformation rates. Dissolved hydrogen concentrations below 0.11μM in sulfate reducing systems resulted in slower rate of PCP degradation. Interestingly, following the addition of a specific inhibitor of sulfate reduction the suppression in PCP transformation rate was relieved (Madsen and Aamand, 1991). The increase in transformation rates following the addition of the specific inhibitor suggests that hydrogen was consumed by the consortium for PCP reductive dechlorination.

In anaerobic PCP degrading serum bottles using imitation vanilla flavoring as an electron donor, PCP was degraded by reductive dechlorination. PCP was exclusively degraded through sequential reductions at *ortho* carbon atoms producing 3,4,5-TCP. With time, 3,4,5-TCP accumulated in all serum bottles. Although 3,4,5-TCP removal was observed, it was considerably slower than the rate of production from PCP reductive dechlorination (Cole and Woods, 2000c; Cole and Woods, 2000d). It was hypothesized that the serum bottles were hydrogen limited which, resulted in the accumulation of 3,4,5-TCP in the system. In an effort to determine the relationship between 3,4,5-TCP reductive dechlorination and hydrogen partial pressure, laboratory experiments were conducted with the following objectives: (i) to evaluate the potential of 3,4,5-TCP reductive dechlorination when hydrogen is supplied as an exogenous electron donor, (ii) to estimate the threshold hydrogen concentration requisite for active 3,4,5-TCP reductive dechlorination, (iii) to investigate the pathway of 3,4,5-TCP degradation and (iv) to examine the potential for the use of hydrogen in a field based remediation scheme.

MATERIALS AND METHODS

Reactor System

Degradation experiments were conducted in a 2.5 L batch reactor maintained at 14°C. The reactor system was fabricated using a section of Kimax glass process beaded pipe (6"x 10" diameter). Stainless steel endplates were sealed to the pipe section using Teflon faced rubber gaskets and compression rings. Tylan mass-flow controllers were used to supply a user-defined mixture of three gasses. Gas transfer to the liquid reactor contents was accomplished with a submerged stainless steel sparging stone. Positive pressure in the rector headspace was relived by a Swagelock check valve installed in the reactor top plate. Two water traps placed in series were used to minimize back diffusion of oxygen into the reactor through the headspace vent valve. Samples were collected through ball valves, which, in normal operation remained closed. The reactor top plate was modified for the airtight installation five electrodes. Using a single

common reference, a pH electrode and a pair of platinum electrodes were used to monitor reactor environmental conditions. Data was automatically logged by an interlaced PC, which monitored the reactor operation. The computer monitored feedback controlled reactor system included several additional features not used during the course of this experiment. Reactor contents were stirred continuously using a magnetic stir plate assembly. A complete description of the reactor system and associated process control features is provided elsewhere (Stuart, 1996).

Inoculum

Consortia used in the serum bottle assay were originally harvested from the pilot scale permeable barrier reactor system installed at the L.D. McFarland facility in Eugene, Oregon. The organisms were used in two previous laboratory studies in which the accumulation of 3,4,5-TCP from PCP degradation was observed. Five pairs of serum bottles containing viable chlorophenol degrading organisms were homogenized in an anaerobic glove box (Coy Labs Grass Lake, MI). The mixture of cells and 3,4,5-TCP was then used to fill and inoculate the 2.5 L batch reactor. The reactor assembly was sealed and purged with nitrogen before installation in the constant temperature chamber. All degradation experiments were conducted at 14°C.

Laboratory Sampling Procedure

Liquid contents of the reactor system were sampled frequently to monitor chlorophenol and anion concentrations. Reactor samples were collected using a glass ground luer lock syringe (Popper & Sons, Hyde Park, NY). Approximately 700 μl of the reactor sample was separated for chlorophenol analysis and was dispensed into glass lined polypropylene eppendorf tubes. The sample balance (ca. 1300 μl), slated for anion measurement, was dispensed to an unlined centrifuge tube. The sample pair was centrifuged at 10,000 RPM for 10 minutes. Duplicate 100 μl chlorophenol samples were collected from the glass-lined tubes and were prepared immediately for chromatographic analysis. Headspace gas composition was sampled using a pressure-lock gas tight syringe (Dynatech Precision Sampling Corp., Baton Rouge, LA).

Analytical Procedures

Aqueous chlorophenol samples were acetylated, extracted into hexane and analyzed as derivatives by capillary gas chromatography. The method was a modification of procedure developed by ((NCASI), 1981; Voss et al., 1980). Extractions were conducted as follows: 500 µl of a solution containing 30.4 g/L K_2CO_3 and 250 µg/L 2,4,6 tribromophenol was combined with a 100 µl chlorophenol sample in a disposable glass culture tube with a Teflon® faced cap. 100 µl of acetic anhydride was added and the tube was mechanically shaken for 20 minutes. 1 ml of chromatographic grade hexane was added and the tube was shaken for an additional 20 minutes. Hexane was removed from the tube and transferred to a 2 ml amber glass vial. The vial was sealed with a Viton® faced crimp cap. Vials were immediately loaded for chromatographic analysis.

Chlorophenols were quantified on a Hewlett Packard 6890 gas chromatograph. Automated 1 µl injections were made on the inlet, which was operated, in a splitless configuration. A Hewlett Packard Chemstation handled signal acquisition and processing from the ^{63}Ni Electron Capture Detector (ECD). Chlorophenol congener separation was accomplished using the following temperature program: initial temperature of 45°C hold 2 minutes; ramp one 25°C/min to 140°C hold 5 minutes; ramp two 5°C/min to 250°C hold 10 minutes. Compound separation was enhanced by a DB-5 (30m x 320µm I.D. x 0.25µm film) fused silica capillary column (J & W Scientific, Folsom, CA). Helium at 35 cm/s served as the column carrier gas. The inlet temperature was 250°C while; the detector was operated at 350°C. A 95/5 blend of argon/methane supplied at 75 ml/min was used for detector make-up. Headspace gas composition was measured with a Hewlett Packard Model 5890 Series II gas chromatograph and a thermal conductivity detector. Separation of gas components was aided by a stainless steel packed column (4'x 1/8" 13x 45/60 molecular sieve). To provide the greatest sensitivity to hydrogen, argon was used as the instrument carrier gas.

Measurement of selection anions in the reactor system was accomplished with a Dionex 2000I Ion chromatograph and a conductivity detector. Anion separation occurred on an Ionpac® AS4A column with a 1.8 mM carbonate/1.7 mM bicarbonate eluant. Laboratory carbonaceous oxygen demand (COD) measurements were conducted using a dichromate digestion procedure. Two-ml samples were placed in commercially prepared tubes, Hach (Loveland, CO) and digested at 150°C for two hours. Sample measurement followed using a Hach DR 890 colorimeter. Solids concentrations in the batch reactor system were analyzed for total and suspended solids using standard methods 2540D and 2540E (Association, 1989).

Chemical Sources

3,4,5-Trichlorophenol (purity > 99%) was obtained from Ultra Scientific Inc., (North Kingston, RI) and was used without further purification. Individual components of the imitation vanilla flavoring were obtained from Aldrich Chemical Co. (Milwaukee, WI). All were reagent grade and possessed purity greater than 99%. Sodium salts of sulfate, nitrate, chloride, acetate and formate used as anion standards were obtained from Mallinckrodt Co., (Paris, KY). The analytical internal standard, 2,4,6-tribromophenol and authentic chlorophenol congeners were obtained from Ultra Scientific Inc.

RESULTS

The effect of hydrogen partial pressure on the reductive dechlorination of 3,4,5-TCP was evaluated in a batch reactor system. The hydrogen partial pressure was varied in an effort to determine the threshold concentration required for 3,4,5-TCP degradation. Changes in the overall reactor environmental conditions, represented by pH and apparent E_H were monitored with time. No effort was made to control

either pH or apparent E_H in the system, values represent the natural system response to hydrogen addition. The temperature during the course of this experiment was representative of aquifer conditions and remained constant at 14°C.

Effect of Hydrogen Partial Pressure on 3,4,5-Trichlorophenol Degradation

The removal of 3,4,5-TCP as a function of hydrogen partial pressure is shown in Figure 6.1 and Figure 6.2. Degradation of 3,4,5-TCP was observed immediately following the addition of hydrogen to the reactor. At the highest hydrogen partial pressure investigated (0.12 atm) 3,4,5-TCP removal progressed steadily. At 70 hours, hydrogen concentrations in the reactor were slowly reduced to 0.005 atm. There was no change in the apparent rate of 3,4,5-TCP degradation following the reduction of hydrogen concentrations. To evaluate the effect of the lowered hydrogen partial pressure over the longer term, the system was maintained at 0.005 atm for period of approximately 15 hours. At a headspace concentration of 0.005 atm degradation of 3,4,5-TCP was seemingly unaffected by the decrease in hydrogen supply. Over a five-hour period starting at hour 85, hydrogen concentrations in the headspace were slowly reduced to zero. During the period of electron donor reduction, the rate of 3,4,5-TCP degradation followed the removal of hydrogen from the reactor and slowed dramatically. In the absence of hydrogen addition, degradation of 3,4,5-TCP ceased completely. Over a 24-hour period lacking hydrogen addition, there was no appreciable removal of 3,4,5-TCP from the reactor system.

To ensure the zero rate of 3,4,5-TCP degradation observed in the absence of hydrogen was a function of electron donor supply and not an effect of other substrate limitations, an aqueous spike of 3,4,5-TCP was added. Concentrations of 3,4,5-TCP in the reactor system increased from 0.28 to 2.45 μM. Following the spike, hydrogen supply to the reactor headspace was withheld for 22 hours. There was no evidence to suggest the biological removal of 3,4,5-TCP at (0.28 μM or 2.45μM) in the absence of hydrogen injection to the reactor. At 135 hours, hydrogen was returned to the reactor. Headspace concentrations were slowly increased in an effort to determine the minimum hydrogen concentration required for 3,4,5-TCP degradation. A transient increase in hydrogen from zero to 0.007 atm was unable to stimulate 3,4,5-TCP degradation. However, as hydrogen concentrations in the headspace decreased, a reduction in 3,4,5-TCP concentrations was noted. Active degradation of 3,4,5-TCP was observed for a period of 22 hours and corresponded to hydrogen concentrations bracketed by 0.0003 atm and 0.00006 atm.

The degradation of 3,4,5-TCP stopped at 0.00006 atm. These results suggested the minimum hydrogen concentration for 3,4,5-TCP reductive dechlorination was between zero and 0.00006 atm Assuming equilibrium partitioning conditions existed in the reactor system this range of hydrogen partial pressures corresponded to an aqueous hydrogen concentration range of 0 to 44 nM. Hydrogen supply was returned to the reactor to reactivate 3,4,5-TCP degradation. However, an increase in hydrogen concentrations failed to stimulate 3,4,5-TCP degradation. Despite hydrogen concentrations previously shown to support 3,4,5-TCP transformation, 120 hours passed before 3,4,5-TCP degradation resumed at

hour 300 (Figure 6.2). The degradation of 3,4,5-TCP corresponded to a hydrogen concentration of 0.03 atm. For the duration of the experiment, 3,4,5-TCP was continuously removed from the system at a constant hydrogen partial pressure of 0.03 atm. Although complete degradation of 3,4,5-TCP in the reactor was not observed, following the concentration spike, approximately 82% of the 3,4,5-TCP present was transformed.

Effect of Apparent Oxidation/Reduction Potential

Degradation of 3,4,5-TCP began immediately after the injection of hydrogen and an associated depression in apparent E_H conditions. The relationship of hydrogen partial pressure to the apparent E_H conditions observed in the reactor are shown in Figure 6.3. In general, the apparent E_H in the system was inversely proportional to the concentration of hydrogen in the reactor headspace. Under the initial experimental conditions, high concentrations of headspace hydrogen helped to depress the apparent E_H conditions. A reduction in hydrogen supply at hour 60 and eventual termination at hour 90, resulted in an immediate increase in apparent E_H of the system. The interaction of apparent E_H on the reductive dechlorination of 3,4,5-TCP is shown in Figure 6.4. Removal of 3,4,5-TCP commenced at approximately 18 hours which, corresponded to an apparent E_H value of –150 mV. Degradation of 3,4,5-TCP occurred between apparent E_H values of –150 mV and –320 mV. Variations in headspace hydrogen composition after 125 hours produced an erratic response in the reactor system. However, apparent E_H measurements never rose above –150 mV. Corresponding with 3,4,5-TCP degradation a sharp decrease in apparent E_H measurements was observed. Despite apparent E_H measurements previously shown to support 3,4,5-TCP transformation, 120 hours passed before 3,4,5-TCP degradation resumed at hour 300. Degradation of 3,4,5-TCP at 300 hours corresponded to an apparent E_H measurements of –220 mV.

Observed Transformation Pathway

Based upon the distribution of chlorinated phenolic compounds in the system, degradation of 3,4,5-TCP was initiated through the removal of the *para* substituted chlorine atom. The reductive dechlorination of 3,4,5-TCP resulted exclusively in the production of 3,5-dichlorophenol (3,5-DCP). Figure 6.5 summarizes the observed distribution of chlorophenols in the batch reactor supplied hydrogen. Production of 3,5-DCP however was not stoichiometric with respect to the initial mass of 3,4,5-TCP present in the reactor. It is likely that 3,5-DCP degradation was occurring coincident to 3,4,5-TCP transformation. Degradation of 3,5-DCP by reductive dechlorination would result in 3-chlorophenol. Production of 3-chlorophenol was never observed. However, the inability to accurately account for total chlorophenol mass through the measurement of metabolic products suggests that continued degradation of 3,5-DCP was occurring.

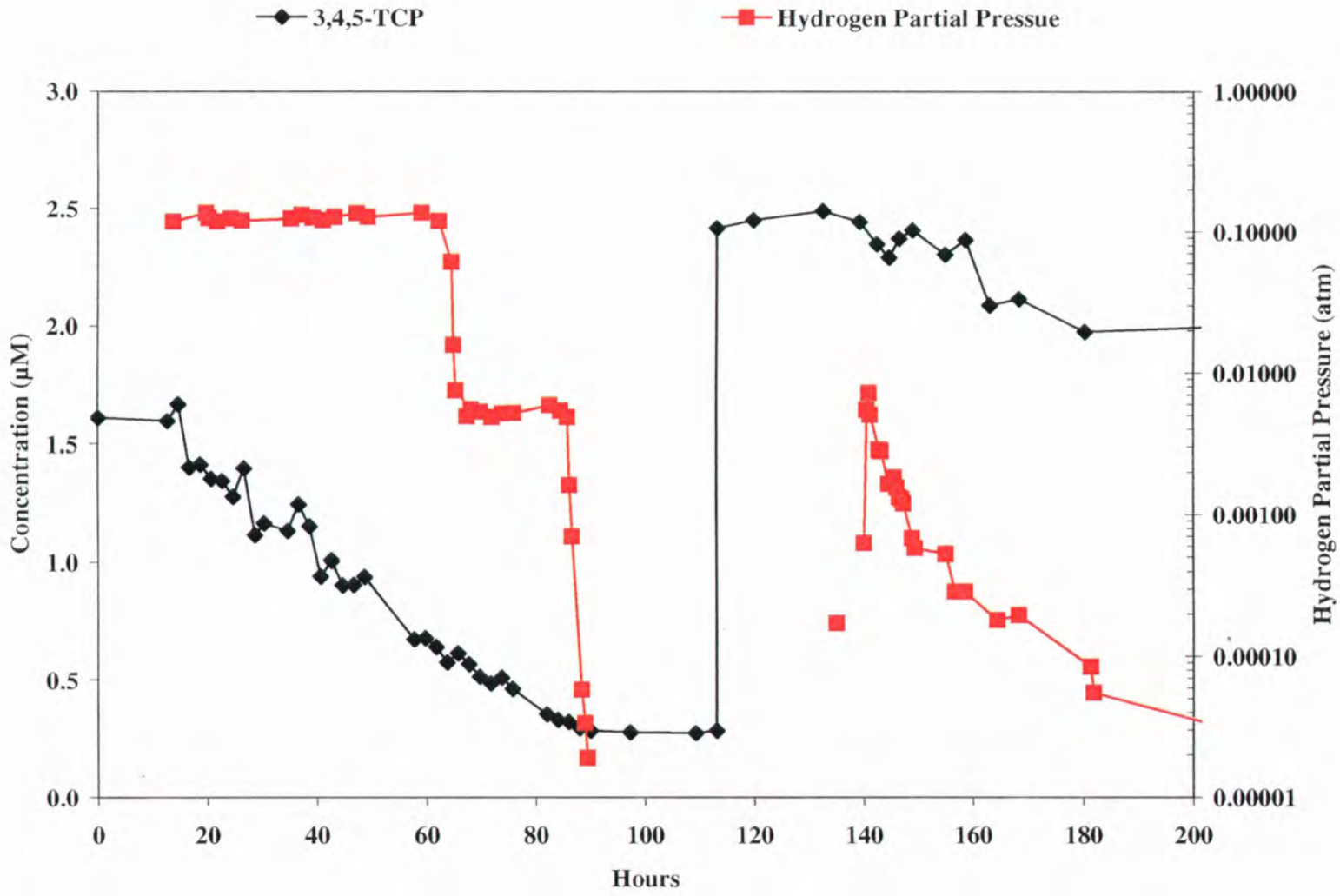

Figure 6.1 The effect of hydrogen partial pressure on 3,4,5-TCP reductive dechlorination (short term)

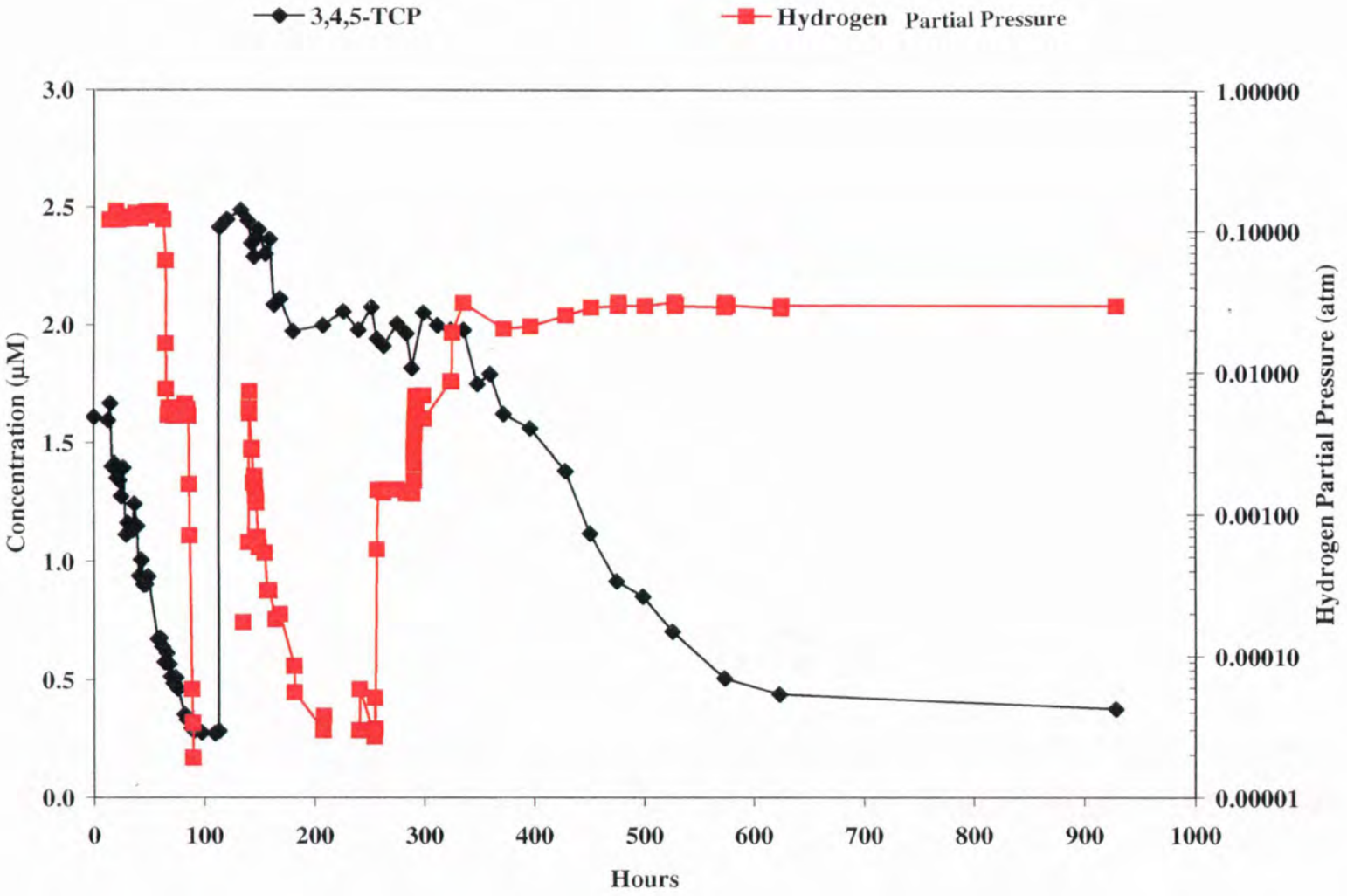

Figure 6.2 The effect of hydrogen partial pressure on 3,4,5-TCP reductive dechlorination (long term)

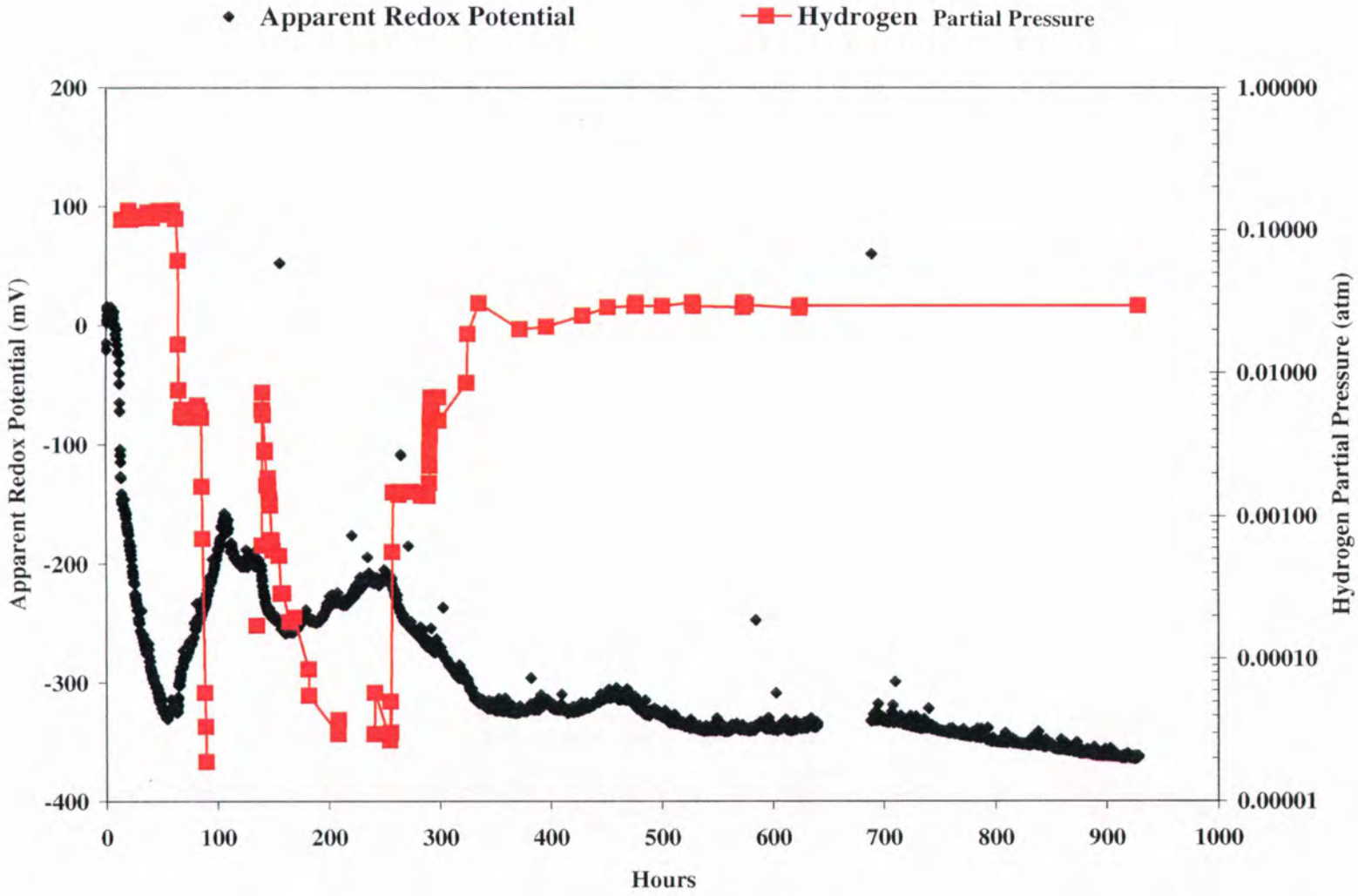

Figure 6.3 Apparent redox potential (E_H) as a function of hydrogen partial pressure

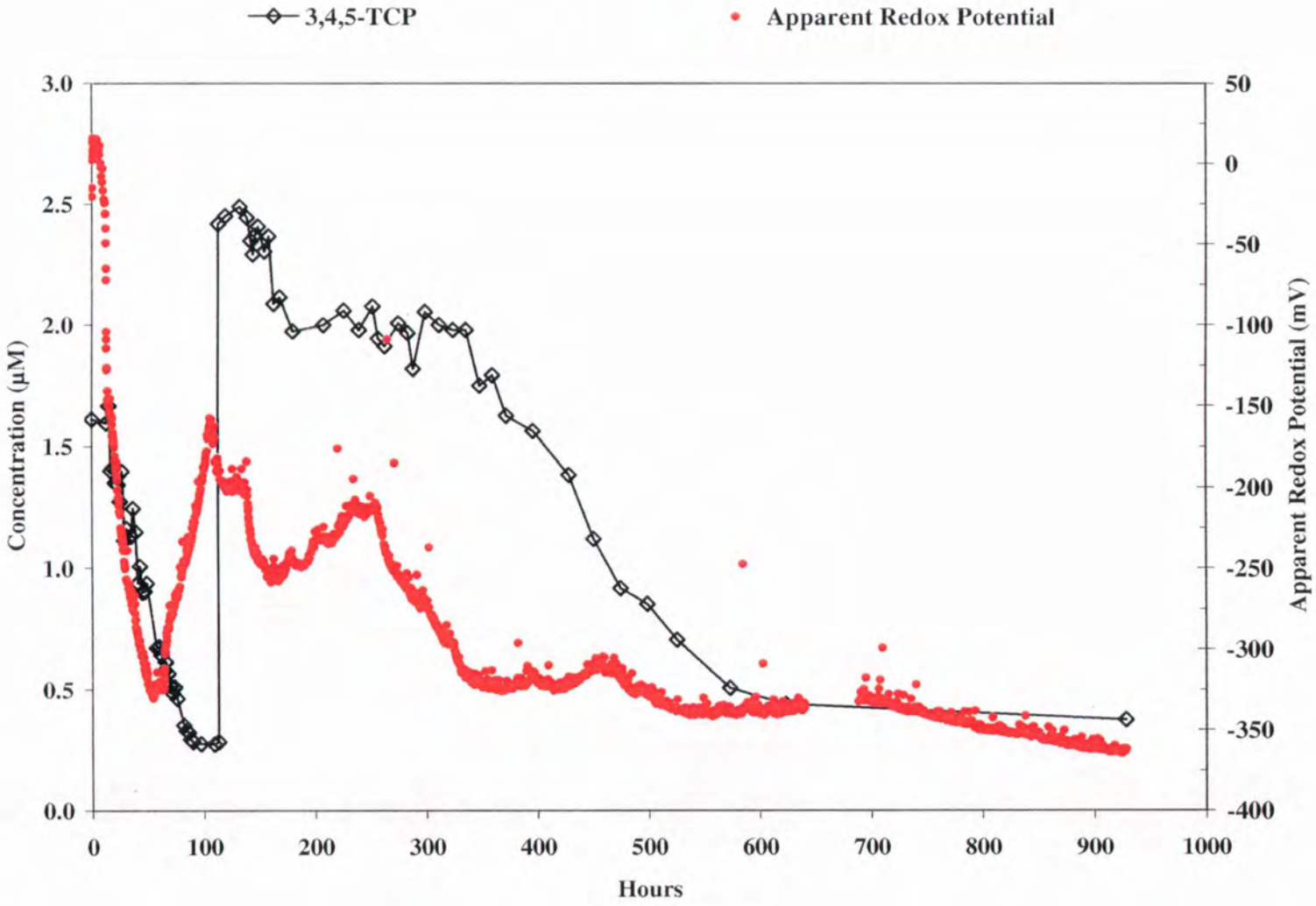

Figure 6.4 Degradation of 3,4,5-TCP as a function of apparent redox potential (E_H)

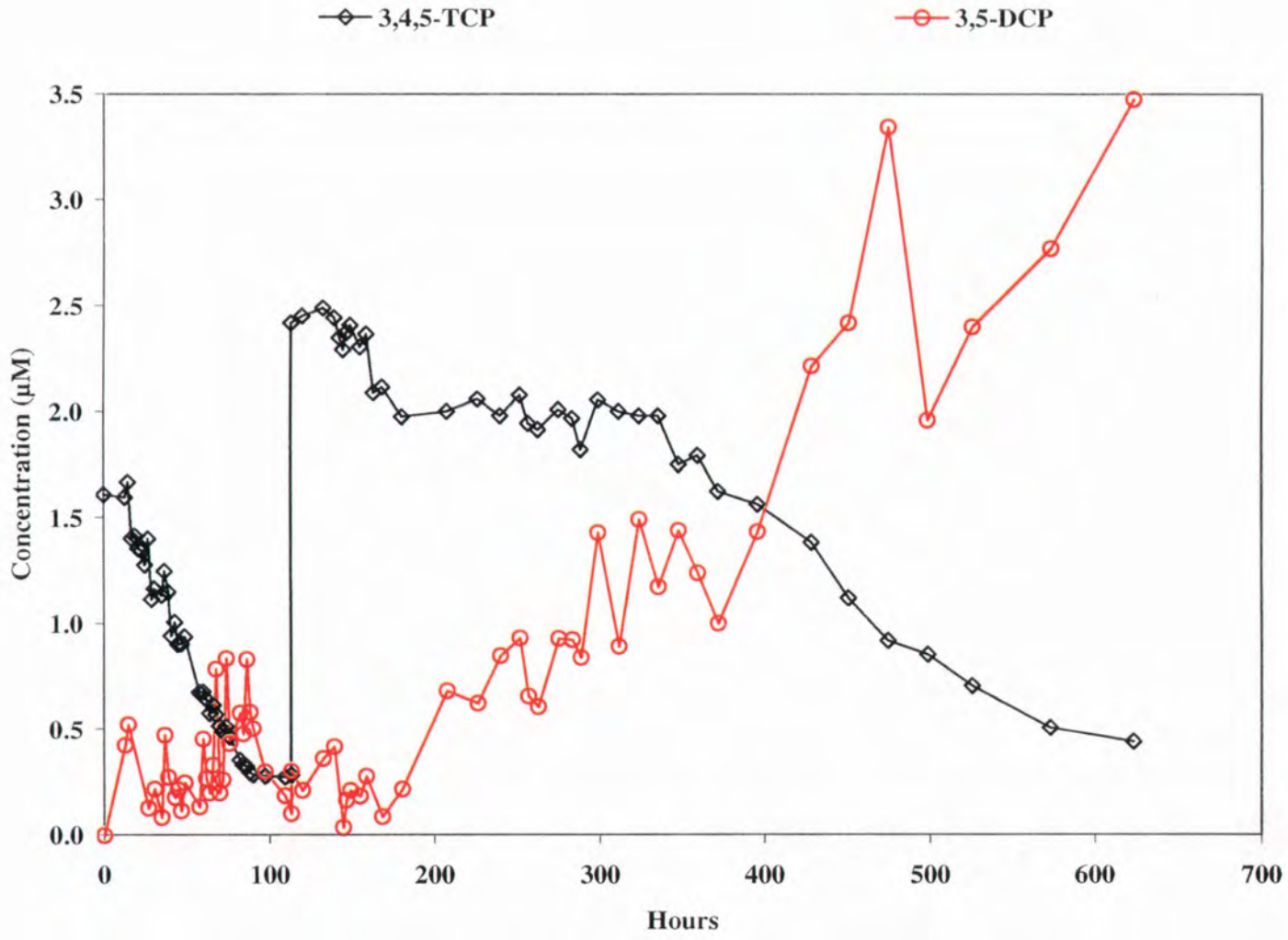

Figure 6.5 Chlorophenols observed in a hydrogen fed batch reactor

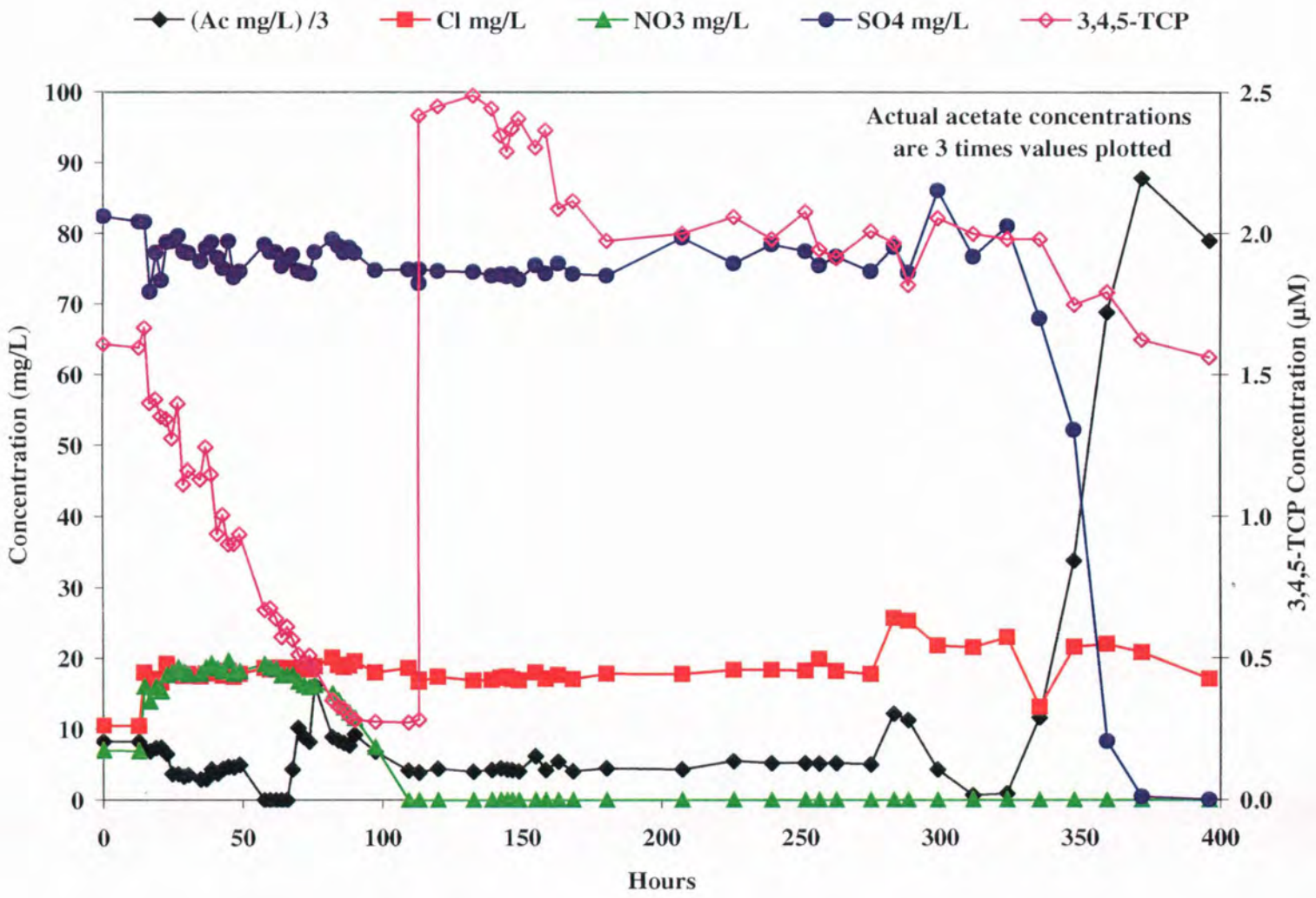

Figure 6.6 Anions observed in a hydrogen fed batch reactor

Effect of Competitive Electron Acceptors

Anions and 3,4,5-TCP concentrations measured during the first 400 hours of the reactor operation are presented in Figure 6.6. To avoid graphical data compression, actual acetate concentrations were divided by three before plotting. All other species reported reflect actual measured concentrations. In the reactor, active removal of 3,4,5-TCP was observed despite the presence of two competitive electron acceptors: nitrate and sulfate, which were initially, present in the inoculum. No appreciable change in nitrate concentration was observed until hour 60, which marked the onset of nitrate reduction. Complete removal of nitrate was observed. From 60 to 109 hours nitrate removal occurred concomitantly with 3,4,5-TCP reductive dechlorination. Sulfate reduction lagged significantly relative to nitrate reduction. Complete removal of sulfate was observed from 325 to 475 hours. The presence of sulfate had no effect on 3,4,5-TCP removal during the first 180 hours of study.

Removal of sulfate from the reactor system corresponded with the observation of increasing acetate concentrations. From system startup to 325 hours, acetate concentrations showed variation but hovered between 12 and 15 mg/L. After 325 hours, a sharp increase in acetate concentrations was observed. Acetate concentrations climbed steadily before reaching a maximum value of 265 mg/L. Prior to the production of acetate at 325 hours, sulfate concentrations in the reactor were relatively stable. However, following the production of acetate, sulfate concentrations rapidly decreased. Results suggest sulfate reduction in the reactor system was supported by acetate.

DISCUSSION

In the presence of a sole exogenous electron donor, hydrogen, 3,4,5-TCP was degraded by reductive dechlorination. Conversely, in the absence of hydrogen addition, 3,4,5-TCP was not degraded. The response of the culture to hydrogen addition was immediate (Figure 6.1) and resulted in steady 3,4,5-TCP removal. Furthermore, a reduction in hydrogen concentration by nearly two orders of magnitude failed to show any effect on the rate of 3,4,5-TCP degradation. Hydrogen was supplied to the reactor at partial pressures spanning several orders of magnitude. While many hydrogen partial pressures were shown to support 3,4,5-TCP reductive dechlorination, an optimum value was not precisely determined. Experimental observations however, suggest the minimum hydrogen concentration required for 3,4,5-TCP transformation is between 0.0015 and 0.005 atm.

Performance of the consortium following the spike in 3,4,5-TCP concentration was unpredictable. Hydrogen concentrations previously determined to sustain 3,4,5-TCP degradation no longer seemed adequate. Perhaps factors beyond the hydrogen concentration were responsible for the apparent lack of 3,4,5-TCP degradation following the reactor spike. It is plausible that the apparent lack of the consortium to remove 3,4,5-TCP after the spike in system concentration is an affect of toxicity. However, this

conclusion fails to explain the 3,4,5-TCP removal observed when the hydrogen supply was returned to the reactor at hour 135 (Figure 6.1). Following the spike in headspace hydrogen concentrations, a brief period of active 3,4,5-TCP degradation was observed. As hydrogen concentrations approached zero, the capacity of the consortium to transform 3,4,5-TCP was lost. Evaluation of apparent E_H during this period (Figure 6.4) failed to yield information that could explain system behavior. Apparent E_H measurements made during periods of active 3,4,5-TCP degradation indicated transformations occurred between –150 and –325 mV. Based on previous experimental observations, apparent E_H measurements from 180 to 300 hours were favorable for 3,4,5-TCP degradation. Reactor pH measurements were constant (data not shown) and failed to elucidate any experimental anomalies that could explain the performance of the consortium during this period. System pH measurements averaged 7.6 over the first 400 hours of observation.

In this experiment, hydrogen gas was the only external electron donor supplied to the reactor system. The presence of acetate in the reactor was not surprising as acetate production occurs by many mechanisms in the anaerobic environment. Acetate was never introduced into the reactor therefore; its presence is attributed to biological processes. Acetate concentrations observed in the reactor as a function of hydrogen partial pressure are presented in Figure 6.7. Interestingly, a reduction in hydrogen partial pressure also marked a sharp decline in acetate concentrations. A rapid rebound in acetate measurements followed as hydrogen in the headspace of the reactor approached a new equilibrium concentration. Presumably, acetate was formed biologically in the reactor system through the coupling of hydrogen and carbon dioxide by homoacetogens; a process known to occur in highly reduced anaerobic environments (E_0 > -290 mV) (Brock and Madigan, 1991).

In the reactor system, ideal conditions for methanogenesis also existed. However, headspace analysis only indicated the presence of methane twice during the experiment. Although a mixture of hydrogen, carbon dioxide and nitrogen were supplied to the reactor, gas flow rates were held constant at 50 ml/minute. The gaseous supply of electron donor provided excellent control of the hydrogen delivered to the reactor. Unfortunately, gas injection into the bulk reactor liquid likely stripped any biologically produced volatile compounds. Thus, the apparent lack of methane was likely a result of continuous liquid stripping rather than limitations of the consortium investigated. Bacterial energetics may have also been responsible for the apparent lack of methane production. Given hydrogen and carbon dioxide, the formation of acetate by homoacetogenesis is more favorable energetically than the methanogenesis (Brock and Madigan, 1991).

It has been proposed that acetogenic bacteria are closely related to the process of reductive dechlorination (Perkins et al., 1994; Zhang and Wiegel, 1990). Therefore, the reductive dechlorination of 3,4,5-TCP may be related to the observation of acetate in the reactor system. Acetate concentrations relative to 3,4,5-TCP removal are presented in Figure 6.8. During the first 50 hours, acetate was removed from the system concurrent to the reductive dechlorination of 3,4,5-TCP. From 110 to 300 hours, acetate

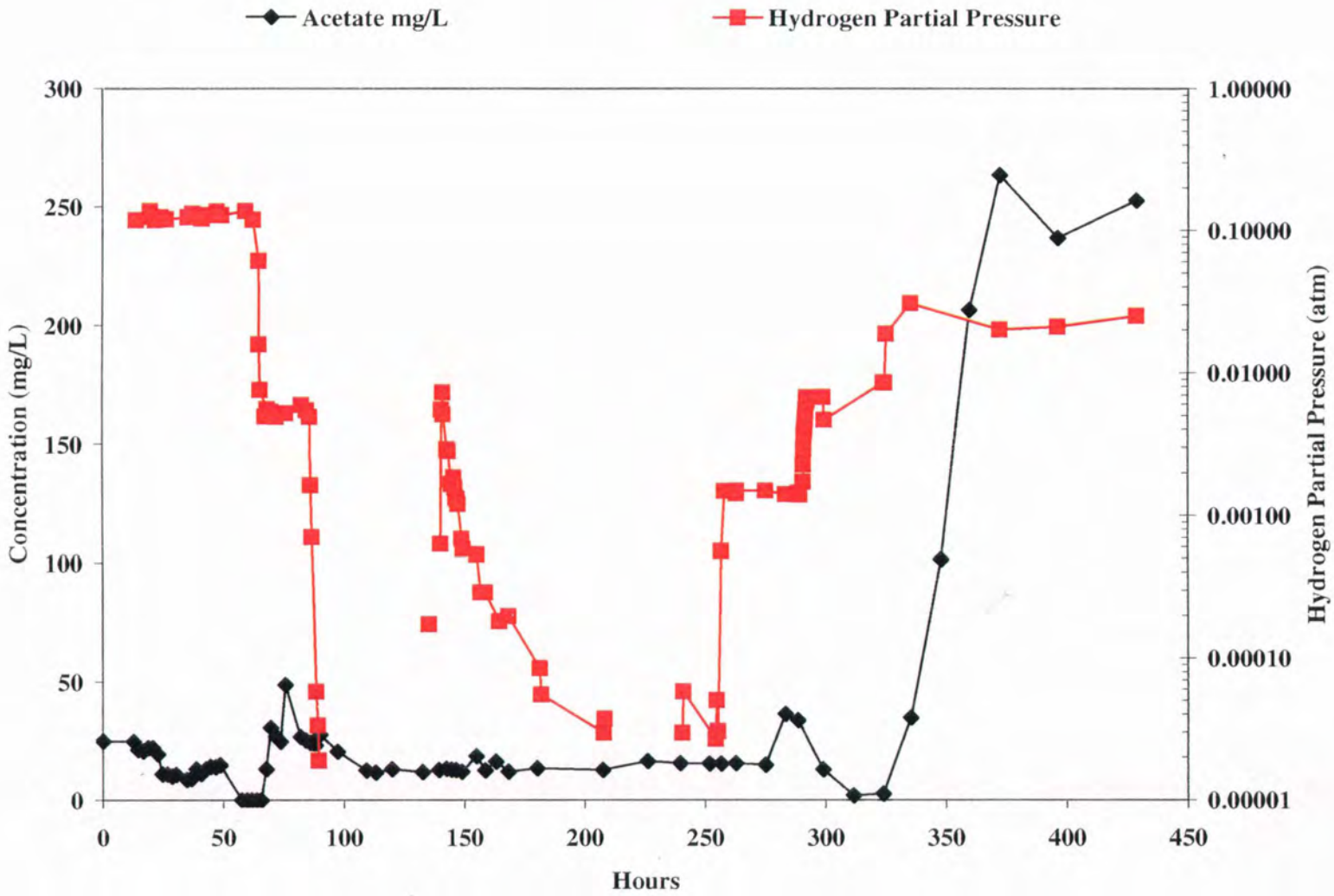

Figure 6.7 Acetate concentrations as a function of hydrogen partial pressure

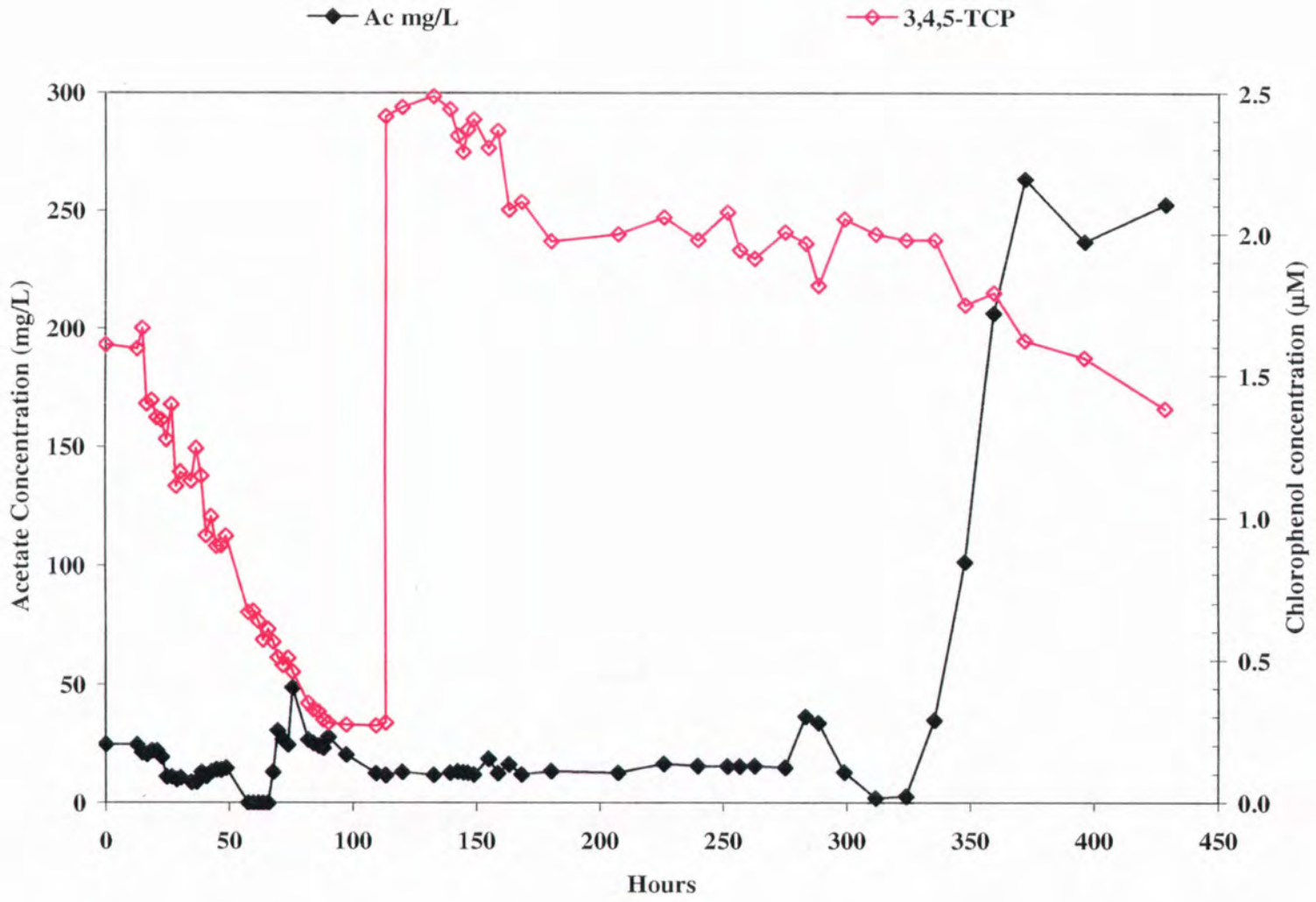

Figure 6.8 Acetate concentrations as a function of 3,4,5-TCP concentrations

concentrations in the reactor system remained unchanged. Suppression of acetate formation and methanogenesis likely occurred as a result of the spike in 3,4,5-TCP concentrations at hour 113. The toxicity of 3,4,5-TCP to acetogens and methanogenic bacteria is well-documented (Bryant et al., 1991; Madsen and Aamand, 1992; Mikesell and Boyd, 1986; Woods, 1985). It is possible that acetate alone served as the electron donor for the reductive dechlorination of 3,4,5-TCP. Acetate is an effective electron donor and many have reported its ability to meet the energetic needs of the anaerobic consortium capable of reductive dechlorination (Woods et al., 1989; Kennes et al., 1996; Chang, 1998; Nicholson et al., 1992).

Acetoclastic methanogens are reported to produce significant amounts of hydrogen in the conversion of acetate to methane (Krzycki et al., 1987; Lovely and Ferry, 1985; Phelps et al., 1985). Perhaps endogenous hydrogen produced from acetate degradation served as the electron donor for 3,4,5-TCP reductive dechlorination. The concurrent removal of acetate and 3,4,5-TCP observed in the first 125 hours and after 300 hours support this conclusion. The relationship of acetate consumption to 3,4,5-TCP reductive dechlorination is further supported by the apparent lack of biological activity observed between 183 and 300 hours. The hydrogen partial pressure maintained in the reactor during this period was previously shown to support 3,4,5-TCP degradation. Yet, if exogenous hydrogen was in fact the only true electron donor, 3,4,5-TCP degradation should have been observed during this period.

While the exact nature of the culture in the reactor system will never be known it is likely a syntrophic arrangement of acetogens, methanogens and dechlorinators existed. The slow decrease in acetate concentration suggests that acetate may have been a common intermediate among the consortia. At the high partial pressures initially evaluated, acetate was likely formed by homoacetogenic bacteria. Acetate concentrations were in turn held at a quasi-steady state through consumption by another member of the consortium. This conclusion is supported by the rapid change in acetate concentrations that followed the changes in hydrogen partial pressure (hour 62) as the homoacetogenic bacteria reestablished steady state acetate production under a different hydrogen concentration (Figure 6.7). Immediate removal of acetate following the spike in concentration observed at hour 75 supports the existence of an active acetate sink in the reactor system. Hydrogen endogenously produced in the batch reactor by acetoclastic methanogens may augment the available electron donor pool or it may act as a requisite condition for reductive dechlorination. Stuart and Woods recently reported the reductive dechlorination of PCP through endogenous hydrogen production by acetoclastic methanogens (Stuart and Woods, 1999).

Removal of 3,4,5-TCP occurred by reductive dechlorination and resulted in the production of 3,5-DCP (Figure 6.5). Concentrations of 3,5-DCP increased with increasing time but were not stoichiometric with respect to 3,4,5-TCP removal. Construction of a mass balance around the chlorophenol species observed failed to adequately account for the complete mass of 3,4,5-TCP present in the reactor system. Degradation of 3,4,5-TCP through a reductive dechlorination at the *meta* position forming 3,4-dichlorophenol was not observed. These results suggest that degradation through 3,5-DCP was occurring in the batch reactor

system. While 3-chlorophenol was not observed in the reactor system, analytical methods used in this study were not specifically tailored for quantification of monochlorophenols. The removal of 3,5-DCP without monochlorophenol observation was also observed in the field treatment system which served as the reactor inoculum (Cole, 1998).

With hydrogen as an electron donor at standard temperature, pressure and unit activity, Gibbs free energies for the reductive dechlorination of 3,4,5-TCP by the *meta* (-142.3 kJ/mol), and *para* (-156.0 kJ/mol) pathways are reported (Dolfing and Harrison, 1992). Degradation of 3,4,5-TCP in the reactor system followed thermodynamic predictions as the reductive dechlorination at the *para* position resulted in the largest net energy release. Removal of the 3,4,5-TCP *para* substituted position may also have been related to a bacterial mechanism of toxicity reduction. Toxicity assays have demonstrated that removal of PCPs *para* chlorine results in intermediate products less mutagenic than PCP (DeMarini et al., 1990).

The presence of competitive electron acceptors in the reactor system seemed to have little effect on the reductive dechlorination of 3,4,5-TCP over the first 180 hours (Figure 6.6). Acetate production and sulfate reduction observed after 300 hours were closely coupled in the batch reactor. These results suggest that acetate served as an electron donor for sulfate reduction. It is unclear why sulfate concentrations remained unchanged for 300 hours despite measurable acetate concentrations in the reactor. Relative to the rate of 3,4,5-TCP reductive dechlorination, sulfate reduction appears to have been favored. The observation of simultaneous sulfate and nitrate reduction and 3,4,5-TCP degradation indicated that competitive electron acceptors were not inhibitory to reductive dechlorination. These results support observations of PCP reductive dechlorination in sulfate amended systems conducted in our laboratory (Cole and Woods, 2000d) and others (Häggblom and Young, 1990; Masunaga et al., 1996).

SUMMARY AND CONCLUSIONS

The effect of hydrogen on the reductive dechlorination of 3,4,5-TCP was evaluated. In the presence of exogenous hydrogen, 3,4,5-TCP was degraded by reductive dechlorination. These results indicate that an inorganic electron donor, hydrogen can support the needs of an anaerobic consortium capable of reductive dechlorination. The minimum hydrogen concentration for 3,4,5-TCP reductive dechlorination appears to fall between 0.0015 and 0.005 atm. These partial pressures expressed as an aqueous hydrogen concentration places the observed threshold on the order of 1000 nM. In comparison to the 2 nM hydrogen threshold for *cis*-1,2-dichloroethene measured by Yang and McCarty, 1998, the observed hydrogen threshold for 3,4,5-TCP degradation was significantly higher. Further studies are required to closer estimate the minimum hydrogen required by this culture. Observation of a sole metabolic product 3,5-DCP, suggests that reductive dechlorination of 3,4,5-TCP occurred exclusively in the *para* position. A mass balance on chlorophenols in the system, and the lack of stoichiometry observed in 3,4,5-TCP transformation suggests that 3,5-DCP was further degraded.

Production and accumulation of 3,4,5-TCP from the anaerobic reductive dechlorination of PCP is a potential pitfall in the application of a biological remediation system for impacted groundwater. Increased toxicity, solubility and mobility associated with 3,4,5-TCP accumulation detract from the convenience of *in-situ* biological treatment strategies. The factors contributing to the accumulation of intermediate metabolites from the reductive dechlorination of PCP are truly unknown. Therefore, in the application of biological treatment for PCP contaminated media, the potential for product accumulation always exists. Results of this study suggest new methods for biological systems where metabolite accumulation is problematic. The stimulation of 3,4,5-TCP reductive dechlorination through hydrogen addition has great utility in the development of field based treatment systems. The explosive nature and low solubility of hydrogen are less than ideal for its use as the sole electron donor in a full scale biological treatment system. However, when used in conjunction with the vast array of electron donors shown to support PCP reductive dechlorination, hydrogen supply to the treatment system could relieve the stresses placed on the consortium resulting from intermediate product accumulation. The benefits of hydrogen use in a remediation system appear promising. As methods to compensate for the production of undesirable metabolic products improve, the effectiveness and acceptance of biological treatment systems will be fully realized.

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CHAPTER 7

ENGINEERING SIGNIFICANCE

Demonstration of the treatment system incorporated research from three distinct project components: Process Development, Technology Development and the Pilot Demonstration. Research contribution and engineering significance of the individual project components are discussed below and summarized by chapter.

CHAPTER 2 EVALUATION OF IMITATION VANILLA FLAVORING TO SUPPORT THE REDUCTIVE DECHLORINATION OF PENTACHLOROPHENOL

The potential application of imitation vanilla flavoring to groundwater remediation is promising, as all components of the mixture are generally recognized as safe (GRAS) by the FDA. It is envisioned that injection of chemicals GRAS, to an aquifer system, may have wider acceptance among the regulatory community, adjacent property owners and facility management officials. Although many electron donors are capable of supporting PCP degrading cultures, the use of imitation vanilla flavoring in a field scale is advantageous for many reasons. Foremost is the ability of imitation vanilla flavoring to catalyze reductive dechlorination in the *ortho*, *meta* and *para* positions. Metabolites that accumulated in other electron donor systems were biotransformed in the imitation vanilla supported system.

On a cost comparison basis, imitation vanilla flavoring is very competitive with other electron donors. Table 7.1 provides a cost based comparison of several electron donors shown to support PCP reductive dechlorination. The table is based on the cost of a single electron transfer from the donor to a microbial population. The costs presented were based on bulk chemical prices obtained from Fisher Scientific and the state of average carbon charge on the donor. The cost and electron contributions for imitation vanilla flavoring were calculated as the aggregate sum of the individual chemical components.

While imitation vanilla flavoring is not the least expensive electron donor, its price is very competitive. Overall, the use of phenol or methanol as an electron donor would result in long term cost savings for the *in-situ* treatment regime. However, the long term cost savings with these materials in a field based treatment system is questionable. Methanol is an extremely volatile and flammable compound. Accordingly, its use in a treatment system would likely require the addition of safety features (e.g. vapor recovery, spark-free pumps, blowers, etc) not required with another electron donor. The capitol cost and the long-term operation of the additional safety equipment is a factor in the treatment system design and cannot be overlooked. In addition to the regulatory difficulties associated with phenol, it is a corrosive and poisonous material, which requires great care in handling and storage.

Table 7.1 Cost comparison of selected electron donors

Electron donor	Density (g/cm^3)	MW (g/mol)	Cost [1.] ($/L)	Electrons [2.] (e^-/mol)	Hazard	Donor Cost ($/$e^-$)
Acetic Acid	1.050	60.05	23.30	8	acid	0.21
Imitation Vanilla Flavoring	-	-	-	3	none	0.17
Lactic Acid	1.200	90.08	88.60	12	acid	0.55
Methanol	0.791	32.04	2.00	6	flammable	0.01
Phenol	1.058	94.11	13.07	28	acid	0.04

Notes:
Bulk chemical costs obtained from Fisher Scientific.
Electron transfer for complete oxidation to carbon dioxide.

Unlike phenol, imitation vanilla flavoring and it's components are GRAS compounds and would present little if any regulatory burden for use in a field application. Pure imitation vanilla flavoring presents little risk in handling, is chemically stable and non-volatile. Furthermore, its theoretical COD of nearly 25,000 mg/L makes it an ideal electron donor. The concentrated nature of imitation vanilla flavoring makes its use at the field scale economical by a reduction in overall storage requirements and consumption rates. The high COD associated with the pure flavoring presents difficulty for bacterial growth and effectively minimizes biological fouling in bulk storage. Biological fouling of feed storage vessels is not normally an issue at the laboratory scale. However, control of extraneous microbial growth is paramount to controlling long-term operation costs of the field treatment system. The engineering advantages for the use of imitation vanilla flavoring are many. When combined with the ability to support of an anaerobic PCP degrading culture, the use of imitation vanilla flavoring as an electron donor in a field based remediation system shows tremendous potential.

CHAPTER 3 PILOT SCALE DEMONSTRATION OF A PERMEABLE BARRIER TECHNOLOGY FOR THE *IN-SITU* BIOREMEDIATION OF PENTACHLOROPHENOL-CONTAMINATED GROUND WATER

Permeable barriers incorporating reactive media such as zero valent iron are an effective treatment mechanism for groundwater impacted by chlorinated solvents. However, the process is limited in application to other groundwater contaminants. PCP for example is not readily removed through iron-catalyzed abiotic transformations. In the case of PCP and other highly halogenated compounds, a

biological permeable barrier capable of reductive dechlorination would be the most effective method of contaminant destruction. In 1996, a permeable reactive barrier was placed on a United States Coast Guard facility in Elizabeth City, North Carolina. The full-scale treatment system, composed of zero valent iron, measured 60 m x 0.6 m in length and width. The barrier was constructed with continuous trenching equipment to depths up to 8 m. Capitol construction costs for the system were $500,000 of which, 35% accounted for the price of the reactive media (Puls, 1998).

In a biological application of a permeable barrier, the consortium serves as the reducing agent for the groundwater and eliminates the need for iron. Therefore, the iron media could be exchanged with a much less expensive material (e.g. crushed stone). Using the Elizabeth City example, replacement of the iron with a biologically reduced media would result in an overall capital cost savings of approximately $175,000. The replacement of iron with a material of higher porosity would provide two benefits to the biological based system: an increase of groundwater flow and a greater ability to resist clogging. The result in capitol cost savings could potentially be offset by operation costs associated with the biological treatment process. For groundwater contaminants not degraded by metal-catalyzed abiotic processes, installation and operation of biological permeable barrier would result in a significant cost savings over a comparable pump and treat system.

A biological permeable barrier reactor was selected for demonstration at an active wood preserving facility in Eugene, Oregon. The facility began operation in the-mid 1950s and applied PCP in a medium aromatic treating oil to telephone poles. Several process variations over the years occurred but without change in treatment chemicals. Operational practices and several accidental spills resulted in contamination of the underlying aquifer with PCP and its carrier oil a light non-aqueous phase liquid (LNAPL). In September of 1993, subsurface remedial action measures were taken. The interim remedial action measure (IRAM) originally consisted of three recovery wells capable of groundwater and LNAPL recovery. A fourth well was added in October of 1996 to ensure plume containment. Extracted groundwater is treated by adsorption on granular activated carbon. When required, skim pumps remove accumulated LNAPL from the wells. Oil recovered is returned to the treating process.

Several comprehensive geologic studies have been conducted at the facility since the identification of subsurface contamination. Soil borings and well construction logs have identified the aquifer on site is a shallow semi-confined structure comprised of two major geologic units. The upper geologic unit averages 10 feet in thickness and is characterized as a dense yet, permeable clay formation. Underlying the clay and ranging in thickness, are well-sorted sands and gravel. Historical measurements reveled groundwater elevations vary seasonally and range from 5 to 15 feet (1.5 to 4.6 meters) below ground surface (bgs). Figure 7.1 summarizes the boring and well locations at the L.D. McFarland facility (RETEC, 1994).

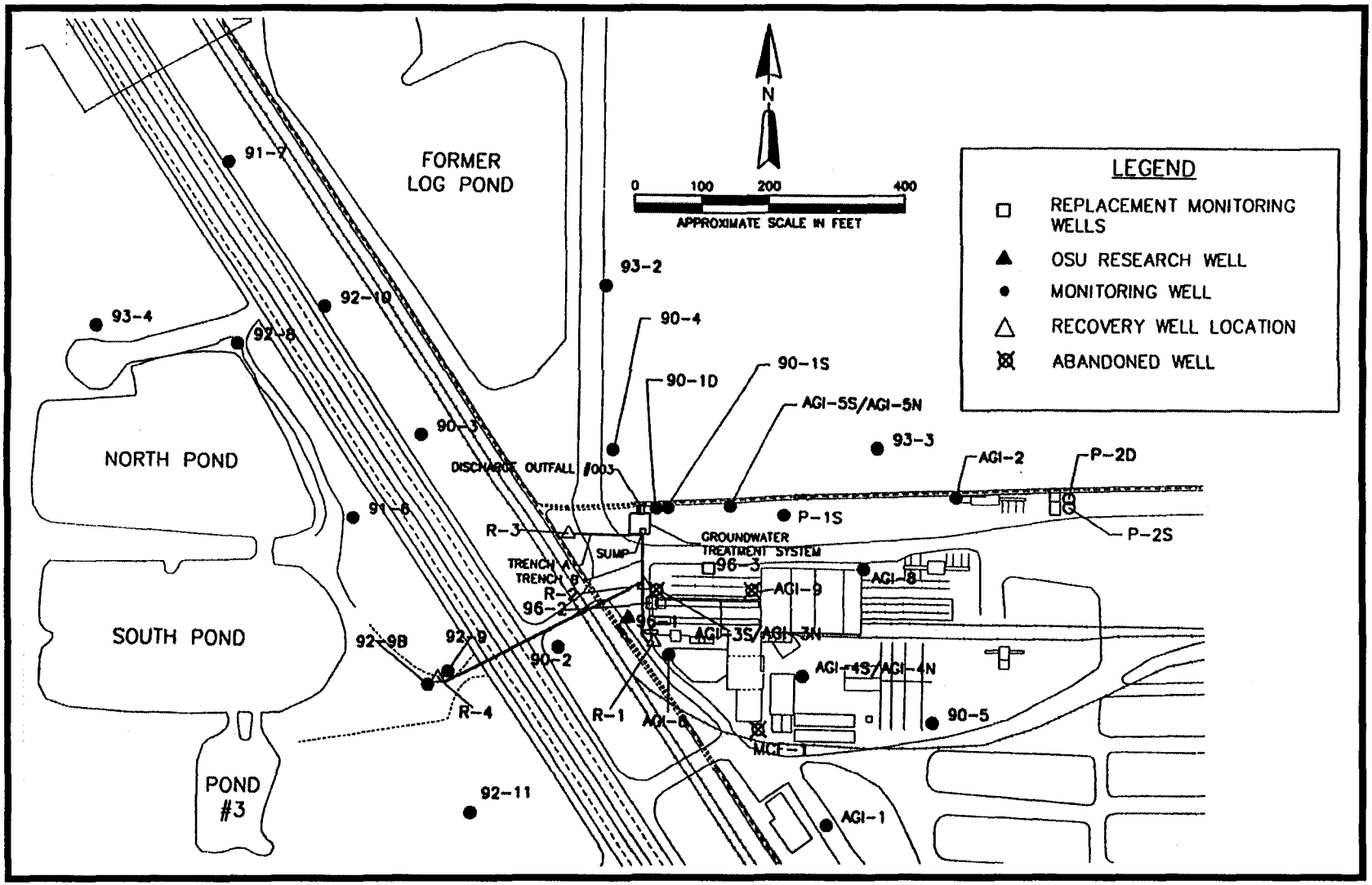

Figure 7.1 Well and boring locations at the L.D. McFarland Facility

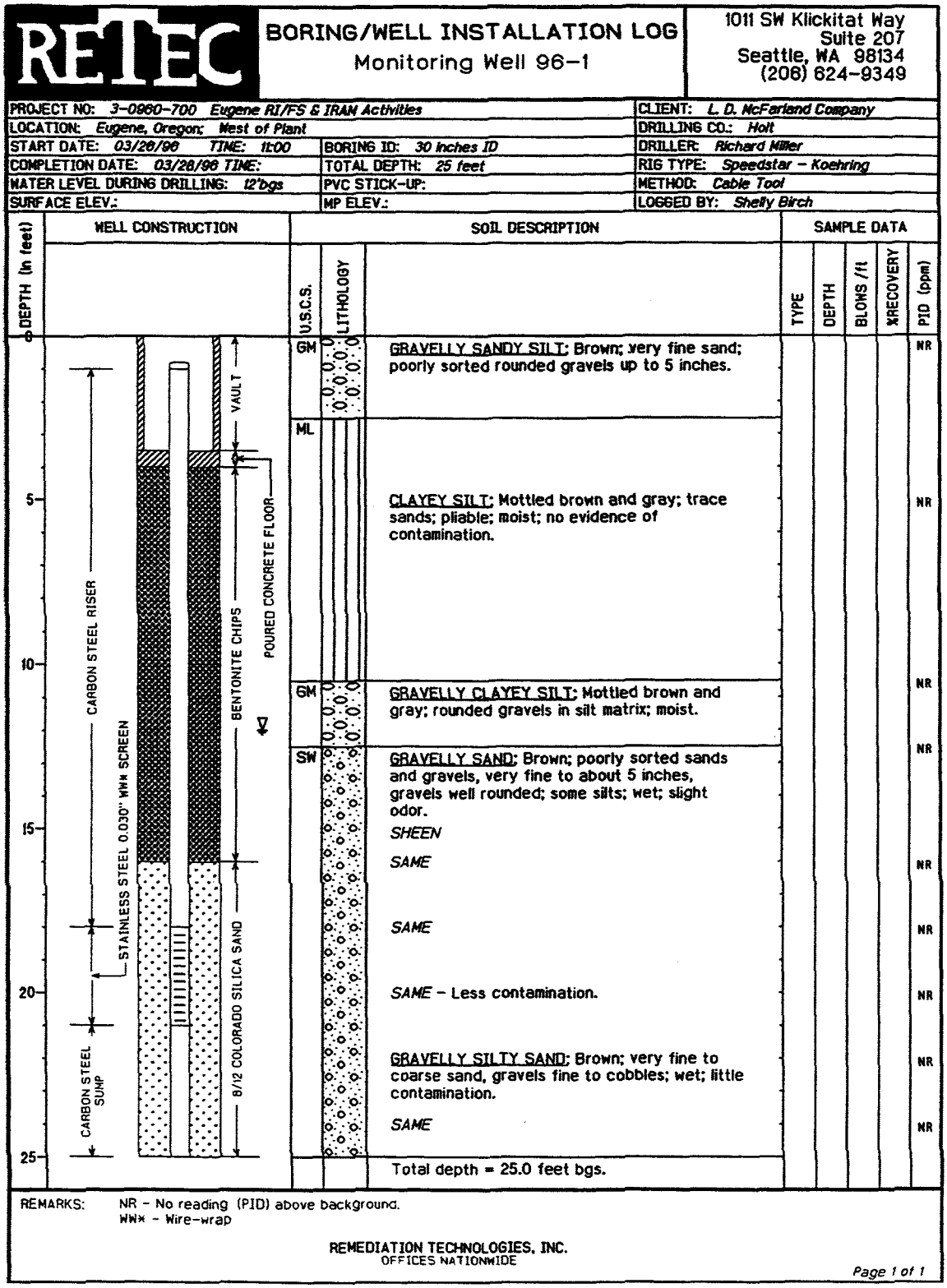

Figure 7.2 Subsurface boring log for MW96-1

The treatment system location was chosen upgradient of two of the site's groundwater recovery wells, which offered complete hydraulic control and excellent accessibility. From a geological perspective, the location was ideal, as the lower confining layer of the aquifer was identified at a minimum distance bgs. Cross sections constructed from boring logs (Figure 7.2) estimated the depth of the lower confining layer at 25 feet (7.6 m) bgs. The shallow aquifer and hydraulic features at the site were ideal for the construction of a permeable biological barrier. Originally, the permeable barrier was proposed in a trench configuration. Unfortunately, the cost of trench construction was excessive relative to the nature of the demonstration project; alternate methods of construction were sought. Ultimately, a "section" of a biological permeable barrier was constructed and designed to fit within the casing of a large diameter well. The experimental configuration offered an economical method to evaluate the effectiveness of a biological treatment regime for PCP contaminated groundwater.

The permeable barrier reactor system was inoculated with a mixture of anaerobic and aerobic sludge, which was harvested from a municipal wastewater treatment plant. Application of the sludge to the reactor system was an effective method of seeding the permeable barrier. However, the Oregon Department of Environmental Quality (DEQ) expressed concern over the pathogenic nature of the sludge used. The DEQ's primary concern was for the protection of surface waters receiving outfall from the IRAM treatment system. It was believed that pathogenic bacteria (coliform) would travel with groundwater to an adjacent recovery well (R2) pass through the IRAM system and impact surface water at the NPDES discharge point. While the scenario was unlikely, the DEQ placed operational restrictions on the technology demonstration for bi-monthly coliform testing and quarterly reporting. Results of the bi-monthly coliform testing program are shown in Figure 7.3. Prior to the reactor inoculation on December 3, 1997, coliform counts varied widely. Coliform measurements at R2 following the reactor inoculation failed to show any response from the permeable barrier operation. There was no evidence to suggest the transport of pathogenic bacteria from the permeable barrier reactor system to the adjacent recovery well during the pilot demonstration. After a 17-month monitoring period, the coliform restriction was removed from the pilot demonstration.

In a full-scale application, it is highly unlikely that pathogenic bacteria would migrate from the biological barrier. The porous nature of the aquifer structure would filter coliform bacteria and retard transport. This process is analogous to coliform removal in water by sand filtration. Furthermore, the temperatures associated with natural groundwater are significantly lower than the optimal temperature for growth of coliform type organisms. In an effort to keep the technology simple, the inoculum used for the permeable barrier was unacclimated wastewater sludge. The sludge had no special treatment prior to use. In the application of a full-scale treatment system, the local wastewater authority will happily fill your material requests at an unbeatable price, gratis. For uniformity purposes, ceramic saddles were used as media within the permeable barrier. The saddles were high in porosity (75%) and surface area. This ensured adequate flow and ample surface for the growth of organisms. While cost prohibitive for use in a full-scale treatment application, uniformly sorted pea gravel would serve the same purpose, but with a

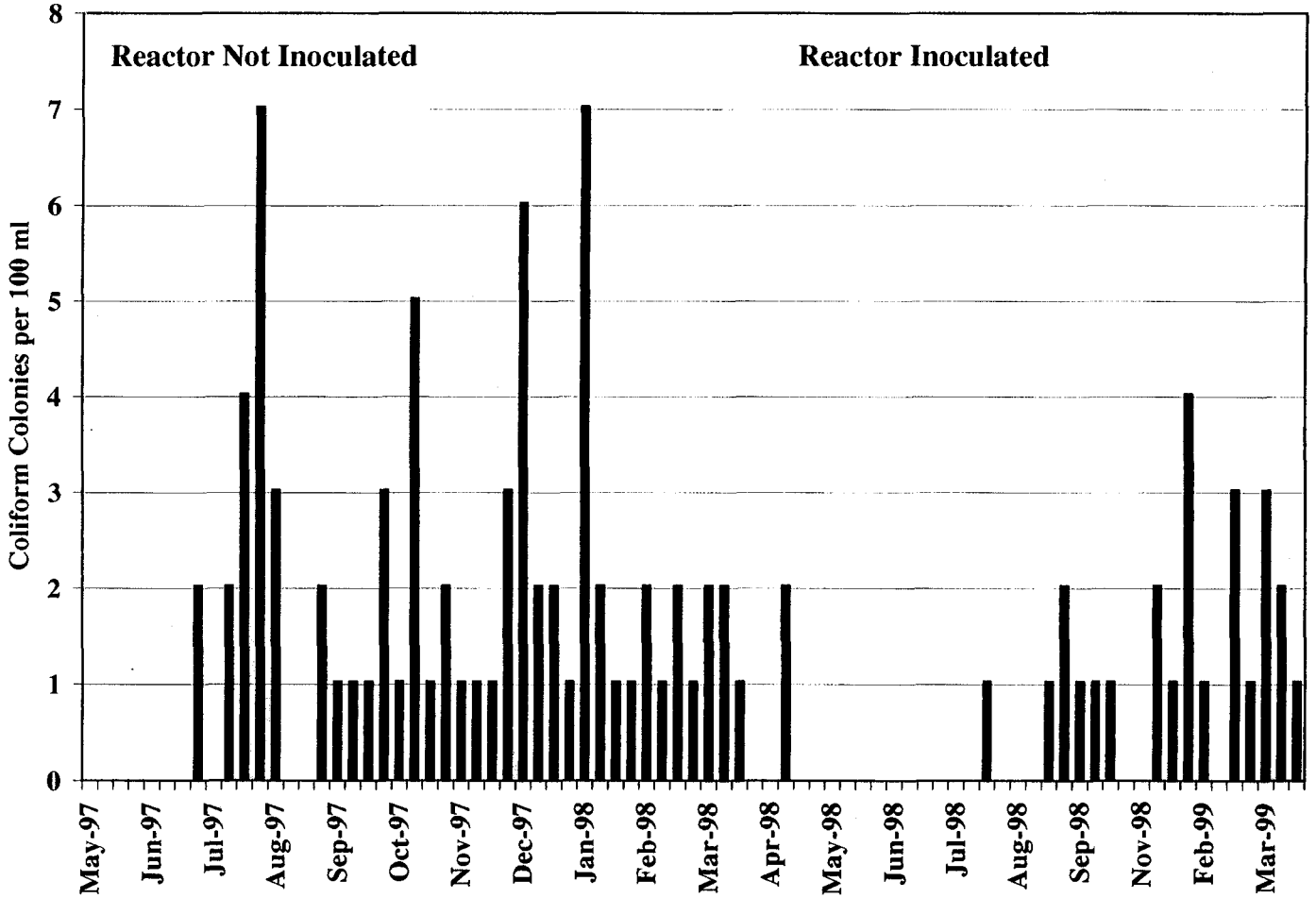

Figure 7.3 Coliform observations down gradient of the permeable barrier reactor

lower surface to volume ratio. The permeable barrier technology used at the L.D. McFarland Site was successful in demonstrating the *in-situ* bioremediation of PCP-contaminated groundwater. Results from the field study complement existing permeable barrier research and expand the applicable range of groundwater contaminants and treatment mechanisms. From conservative tracer studies and PCP concentrations measured in the treatment unit over the demonstration, the flux of PCP traveling through the unit was computed. Figure 7.4 represents the cumulative mass of PCP measured in the reactor with time. Labeled zones in Figure 7.4 depict experimental changes in reactor system operation.

During phases I-III, there was no appreciable reduction in the cumulative mass of PCP, indicating the absence of PCP transformation. Process changes during phase IV however, marked a plateau in the mass of PCP present in the system. This plateau in cumulative mass was a result of biological PCP removal in the reactor system. It is clear that the unit effectively mitigated the transport of PCP in aquifer structure. The increase observed in cumulative PCP mass around 700 days was a result of experimental perturbations.

Data collected in the presence and absence of permeable barrier reactor operation was used to construct Figure 7.5, which depicts PCP flux as a function of treatment condition. During the 97-98-field season, represented on the bottom x-axis, the flux of PCP (solid line) through the system was quite variable. The flux of PCP in the system followed a response similar to site groundwater elevations. The response in PCP concentrations followed historical site observations, which corresponded, to seasonal periods of precipitation (November-April). In the absence of treatment, the increase in PCP flux through the treatment system late in 1997 is evident. Early in the 98-99-field season, represented on the top x-axis, active biological PCP removal commenced. The attenuation of PCP flux (dashed line) through operation of the permeable barrier reactor is evident. Comparison of contaminant mass flux in the absence and presence of treatment clearly shows the contribution of the permeable barrier reactor in the treatment of PCP impacted groundwater. Through the comparison of cumulative, PCP mass in the presence and absence of barrier operation, mass removal by the system was computed (Figure 7.6). Operation of the unit over the 97-98-field season (solid line) shows the steady accumulation of PCP mass in the system where as, operation in 98-99 (dashed line) shows a slight increase. Comparison of the system operation over two consecutive years allowed the estimation of PCP removal. Furthermore, comparison over identical time frames eliminated potential bias from seasonal water table variations. At the demonstration scale, it is estimated that the permeable barrier reactor effectively removed approximately 55 grams of PCP from the groundwater. Over a comparable 12 month period, the IRAM system removed a total of 69 Kg of PCP from extracted groundwater and recovered LNAPL (RETEC, 1997; RETEC, 1998).

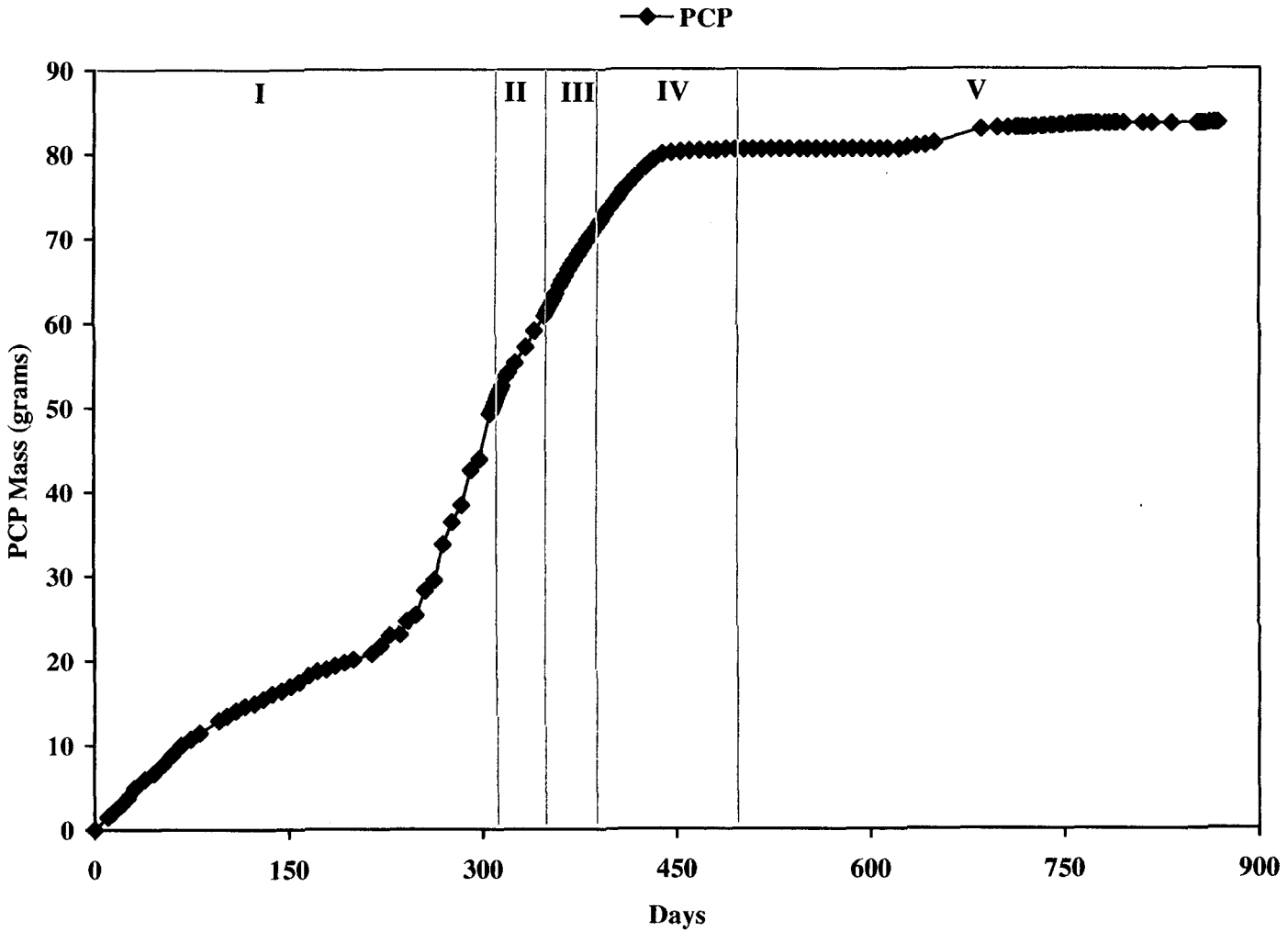

Figure 7.4. Cumulative mass of PCP measured in the permeable barrier reactor

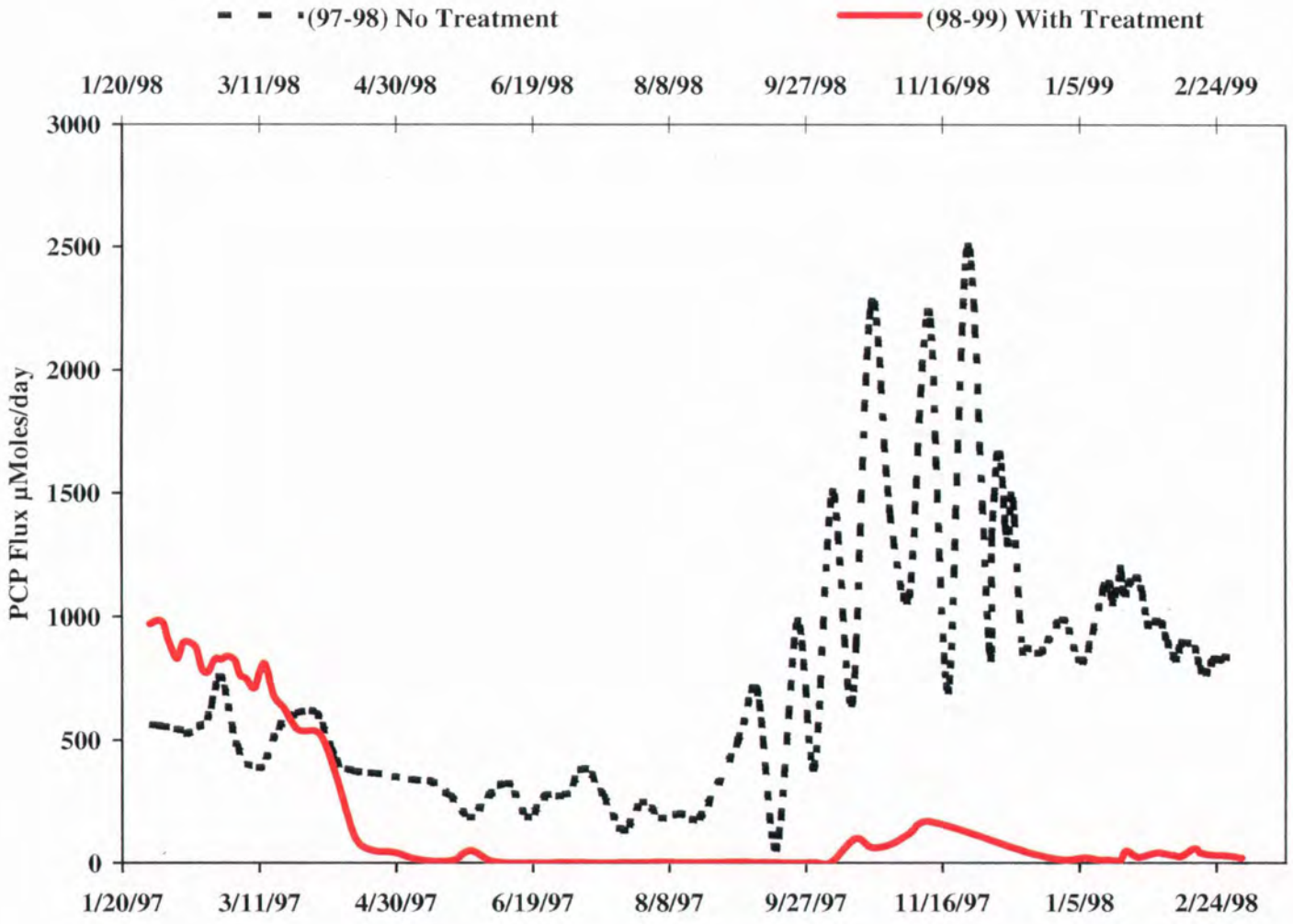

Figure 7.5 Observed impact of treatment system on PCP groundwater flux

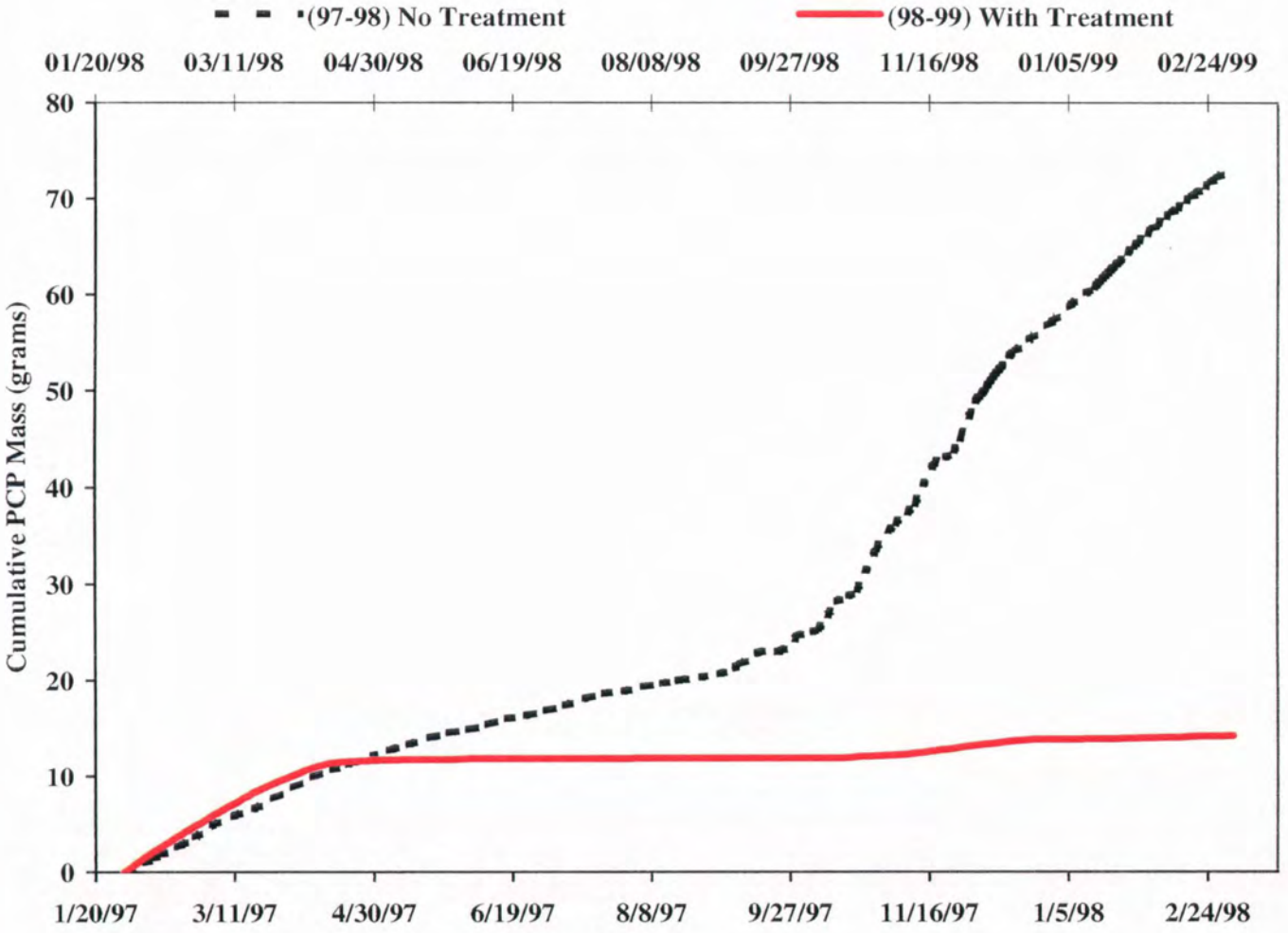

Figure 7.6 Estimated contribution of an in-situ biological treatment system for PCP degradation

Table 7.2 Treatment system operational comparison

Treatment System	PCP Removed	Liters Treated	PCP Removed / Liter Water treated
IRAM-pump & treat	69 Kilograms	92×10^6	0.74
Permeable Barrier	55 grams	73,000	0.75

While the mass of contaminant removed seems small, the overall efficiency of the barrier is comparable to the IRAM system currently in operation on site. Table 7.2 provides an operational summary and comparison of treatment efficiencies for the IRAM system and permeable barrier reactor. When the PCP removed is normalized to the volume of water treated, removal efficiencies in each system are remarkably similar. Operation of the permeable barrier system was estimated to remove 0.75 mg PCP per liter treated. In comparison, the IRAM system removed 0.74 mg PCP per liter treated. It is clear from a normalized comparison of treatment system performance that the biological permeable barrier system was an effective method for *in-situ* groundwater remediation.

While the reactor system demonstrated at the McFarland showed excellent biological PCP removal, the physical system used would be more appropriate in a location with deep subsurface contamination. The construction of large diameter wells is practiced in many areas of civil engineering. As such, the construction methods are reliable for many types of aquifer material and costs are quite reasonable. The McFarland site is an ideal location to explore the potential for the installation of a full-scale biological permeable barrier. Contamination on site is relatively shallow which greatly expands the pool of applicable construction methods. Implementation of such a treatment system would likely require stabilized excavation. Given depths and materials on site, perhaps the excavation could be completed with shoring boxes. Excavation by this method would be considerably less than trench stabilization with sheet piles. The use of continuous trenching equipment would likely be impossible due the heterogeneous nature of the gravel formation on site.

Perhaps the most effective method of full-scale deployment at the McFarland site would be through a modification of the Funnel and Gate system. In the process patented by the University of Waterloo, vertical sheet piles funnel groundwater flow to a common exit or gate where it is treated by zero valent metals. While the process is not applicable for PCP, the physical structure is ideal for a biological treatment system. On the McFarland site, the funnels could be constructed using a variety of subsurface flow control techniques: sheet piles, grout curtains or slurry walls. While the treatment unit at the "gate" is

an *in-situ* biological reactor constructed in the confines of a rectangular sheet pile array or perhaps in a large diameter well. A treatment system constructed in this method would allow process control similar to the demonstration reactor yet; the volume of treated water would be greatly increased.

Treatment systems of any type will always posses a hefty capitol cost to the site owner. Yet, the cost savings associated with long term operation of a biological permeable barrier seem to be significant over conventional pump and treat methods. Yearly operation of the IRAM system at the McFarland site currently run around $40,000. The majority of the operating costs are in the replacement and maintenance of the granular activated carbon adsorption beds. Any treatment technology therefore, that eliminates the need for the granular activated carbon has the potential to reduce long-term operation costs of the IRAM system. Armed with the demonstration results in 1993, the year of IRAM installation, a compelling case for full-scale *in-situ* treatment could have been made. Today, successful operation of the IRAM system and the capitol construction cost associated with a full-scale permeable barrier make the economics of the application questionable. It is clear from a cost and performance prospective that the replacement of the IRAM system with a biological permeable barrier would not dramatically decrease the time required for aquifer restoration. However, installation of a full-scale biological system would serve as a landmark step in the development of biological remediation strategies for impacted groundwater structures.

The successful remediation of complex sites contaminated with waste mixtures will require the use of multiple treatment technologies. Unfortunately, bioremediation technology development has largely focused on strategies for treatment of an individual compound or a closely related group of contaminants with a narrow range of physical, chemical, or biological characteristics. While this demonstration was no exception, the modular design of the permeable reactor allows for the combination of biological and or physical/chemical treatment techniques. The application of a biological treatment strategy for the remediation of chloroaromatic compounds marked a significant deviation from the permeable barrier applications currently in use. The results of this study clearly indicate the potential for the successful remediation of groundwater contaminants in a biological permeable barrier configuration. The success of the biological treatment strategy provides yet another tool for the design engineer to use independently or in conjunction with other remediation strategies.

CHAPTER 4 FIELD AND LABORATORY COMPARISONS OF SUBSTRATE REQUIREMENTS FOR THE BIOREMEDIATION OF PENTACHLOROPHENOL-CONTAMINATED GROUND WATER

Research at the laboratory, bench, pilot, and field scale provides countless examples of the transformation and degradation capacity of biologically mediated treatment systems. Laboratory research has shown what contaminants are amenable to biological treatment while, pilot and field scale projects have demonstrated the most successful application methods. There is an inherent link between the long-term success of the field scale system and fundamental treatment mechanisms derived under the confined

and controlled laboratory environment. The link between the laboratory and field arenas allows an iterative approach to determine the environmental conditions in which a treatment system will adequately transform target components or ultimately fail. Beyond the scientific aspects, combined laboratory and field based studies allow for economic evaluations and optimization of a particular treatment technology.

The optimization and economics of a treatment system are derived from the points in which the treatment system can no longer operate within the ranges of desired efficiency. Scientifically, these points are defined by system failure resulting from the perturbation of components critical to fundamental unit operations. Aside from the success of a biological treatment scheme, what truly dictates the potential application of the technology is capital and operation costs. Capitol costs in any remediation process vary widely and are often out of the engineers control. Once constructed however, flexibility in treatment system operation allows for the control of operation costs. Inherently, the physical nature of a treatment system significantly contributes to operation costs. However, on a comparative basis the operation cost of any biological treatment strategy is directly related to the cost and quantity of required exogenous substrates.

Evaluation of the substrate requirements for PCP reductive dechlorination illustrated the importance of substrate optimization studies. Results of the laboratory study suggested that a 10-fold reduction in the supply of electron donor would not appreciably alter PCP biotransformation. Performance of the pilot scale system following the reduction in substrate concentrations confirmed the laboratory predictions. The study did not truly optimize substrate requirements in the treatment system. However, it did indicate the potential for serious operational cost savings through the substrate supply reduction. It is likely that further reductions in substrate are possible without comprise of the biological process.

Bioremediation technology offers powerful treatment solutions for contaminated soil and groundwater systems. Biological systems are capable of contaminant mineralization at comparably lower capital and operational costs than conventional treatment regimes. Continued research incorporating companion laboratory and field studies will help delineate the boundaries in which the aspects of *in-situ* biological treatment, engineering and economics are combined to develop cost effective solutions for groundwater remediation.

CHAPTER 5 THE EFFECT OF SULFATE ON THE REDUCTIVE DECHLORINATION OF PENTACHLOROPHENOL: A FIELD AND LABORATORY COMPARISON

Reductive dechlorination remains one of the most effective processes for the microbial degradation of highly halogenated aromatic compounds. The ability of a natural electron acceptor like sulfate to inhibit or alter the degradation process has serious implications for the ultimate success of an *in-situ* biological treatment scheme. Generally, the presence of sulfate is shown to inhibit the reductive dechlorination of PCP under laboratory conditions. However, results of laboratory study indicated that under the sulfate

concentrations investigated, transformation of PCP was not affected. Evaluation of PCP reductive dechlorination in the presence of sulfate at the field scale was complementary to laboratory findings. Although not investigated, it is expected that the performance of the reactor in the presence of nitrate would be similar to the sulfate amended system.

In the treatment system evaluated, sulfate concentrations were not high enough to pose a threat to PCP reductive dechlorination. Perhaps the concentration of electron donor was high enough to satisfy the needs of the sulfate reducing and dechlorinating microbial populations. In the presence of increasing sulfate concentrations, PCP degradation would have possible at the expense of increased electron donor supply concentrations. At extremely high sulfate concentrations, representative of estuarine ecosystems, application of biological strategies for reductive dechlorination is questionable. Sulfate reduction is a common process and generally will proceed in the presence of any suitable electron donor. Unfortunately, the biological reduction of high sulfate concentrations can greatly impact the anaerobic community with the toxic effects of sulfide production.

The ability of the pilot scale reactor system to tolerate sulfate and degrade PCP is likely a site-specific condition. Therefore, detailed laboratory studies are suggested prior to the application of this technology to sulfate rich groundwater structures. Application of the permeable barrier reactor to groundwater structures high in sulfate is possible but system and process modifications would be likely. In case of very high sulfate concentrations, pretreatment may be necessary before reductive dechlorination effectively proceeds. Removal of sulfate in pretreatment could proceed by biological or abiotic mechanisms. In a traditional biological treatment regime, stimulation of sulfate reduction would likely occur following the introduction of an electron donor. If iron chloride was injected with the electron donor, precipitation of the biologically produced sulfides could effectively control toxicity to other consortium members. If required, a separate system specific for sulfate removal could be placed up gradient of the permeable barrier reactor. Direct injection of an electron donor mixture containing iron chloride to the reactor treatment zone could suffice in systems were low concentrations of sulfate inhibits reductive dechlorination. Use of an abiotic treatment zone comprised of zero valent iron could effectively transform sulfate and precipitate iron sulfide. Regardless of treatment mechanism, the modular design of the reactor system would have easily allowed process modification to include a pretreatment zone specifically for sulfate removal.

CHAPTER 6 THE EFFECT OF HYDROGEN ON THE REDUCTIVE DECHLORINATION OF 3,4,5-TRICHLOROPHENOL

Accumulation of 3,4,5-trichlorophenol produced though the reductive dechlorination of PCP may have a profound effect on the overall transformation efficiency of an *in-situ* treatment system. In laboratory tests, PCP was degraded by reductive dechlorination in serum bottles using imitation vanilla flavoring as an electron donor. PCP was exclusively degraded through sequential reductions at *ortho* carbon atoms

producing 3,4,5-TCP, which accumulated in all serum bottles. Although 3,4,5-TCP removal was observed, the performance of the chlorophenol degrading system was compromised (Cole and Woods, 2000c; Cole and Woods, 2000d).

In a biological strategy for PCP contaminated groundwater, the accumulation of 3,4,5-TCP is undesirable. Laboratory studies conducted in an anaerobic system where 3,4,5-TCP accumulated suggest that hydrogen addition can stimulate reductive dechlorination. Although 3,4,5-TCP accumulation was not observed in the pilot demonstration, it was observed in serum bottle assays conducted with indigenous microbes and site ground water. The ability of hydrogen addition to stimulate 3,4,5-TCP reductive dechlorination would act as sort of contingency plan in the field. The process configuration used in the permeable barrier reactor would have easily allowed for hydrogen addition had 3,4,5-TCP accumulation occurred. The use of hydrogen in a field scale treatment system certainly has its pros and cons. When supplied as an electron donor, hydrogen has been shown to support the reductive dechlorination of PCP and 3,4,5-TCP. Hydrogen gas is not appreciably soluble and the majority of gas supplied to the system would escape to the atmosphere. The efficiency of the mass transfer is not of dire concern because hydrogen gas is relatively inexpensive. The hazard of explosion and fire on the other hand is severe. The reactivity of hydrogen gas is significant and special precautions are required for safe use. Maintenance of a hydrogen supported remediation system would likely require skilled labor because of the inherent danger associated with the feed stock. A blend of hydrogen and nitrogen would alleviate the fire and handling hazard but it would result in higher operation costs.

Laboratory results indicate that low levels of hydrogen were effective for stimulation of 3,4,5-TCP dechlorination. Based on the low solubility of hydrogen and its associated hazards, development of time release hydrogen system could prove beneficial in the design of biological remediation systems. Chemical products offering this capacity have been developed and marketed by Regenesis Co. Application of zero valent iron in a reduced environment could allow for hydrogen production through accelerated anaerobic corrosion (Matheson and Tratnyek, 1994; Puls, 1998). Small volumes of hydrogen could also be effectively produced *in-situ* though current induced hydrolysis. Modification of the reactor to handle any of the methods of hydrogen delivery would be relatively easy. In a field scale application, the potential method for hydrogen supply would certainly warrant a comprehensive analysis that focussed on operation economics and system safety.

CHAPTER 8

SUMMARY & CONCLUSIONS

TECHNOLOGY SUMMARY

Permeable barriers are a promising technology for remediation of contaminated aquifers. As a remediation strategy, permeable reactive barriers were first proposed by the United States Environmental Protection Agency (USEPA) in 1982. At this time, little interest was placed in the technology development. In 1989 the concept of an *in-situ* reactive wall was revisited and further developed by the University of Waterloo, Canada. This research group is credited with the first full-scale *in-situ* demonstration of a permeable barrier in Borden, Ontario, Canada. By 1998, over 500 studies had been identified which sought to expand and develop this treatment technology. Of these studies, 20 were identified as commercial applications. The growing interest in permeable barriers reflects the competitive operational costs compared to conventional techniques like pump and treat. Recent estimates show a cost reduction of greater than 30% is possible in the application of a permeable barrier system compared to conventional pump and treat (Burmeier, 1998).

To date the development of full scale permeable barrier treatment techniques has focussed on abiotic treatment methods using zero valent metals for groundwater contaminated with inorganic metals and chlorinated organic solvents. In 1996, a permeable reactive barrier was placed on a United States Coast Guard facility in Elizabeth City, North Carolina. The full-scale treatment system, composed of zero valent iron, measured 60 m x 0.6 m in length and width. The barrier was constructed with continuous trenching equipment to depths up to 8 m. Capitol construction costs for the system were $500,000 of which, 35% accounted for the price of the reactive media. In comparison to the proposed pump and treat system, a cost savings of $5 million dollars over a ten-year period was estimated with the permeable reactive barrier installed. The estimated savings related primarily to the difference in long-term operation and maintenance costs between pump and treat and the permeable barrier system installed (Puls, 1998).

The use of reactive media permeable barriers is an effective remediation method for groundwater contaminated by metals and chlorinated solvents. Unfortunately, the application of reactive media technology is limited to groundwater contaminants amendable to abiotic transformations. While a host of physical and chemical transformation mechanisms have shown potential for use *in-situ*, biological processes for groundwater remediation clearly posses distinct advantages. They are naturally occurring, amendable to a wide array of substituted aliphatic and aromatic compounds and generally result in the production of innocuous by-products. In comparison to technologies in which the contaminant is removed by precipitation or adsorptive mechanisms, biological processes are superior. For contaminants not

favorable to abiotic transformations, bioremediation technology offers powerful treatment solutions for contaminated soil and groundwater systems. Biological systems are capable of contaminant mineralization at comparably lower capital and operational costs than conventional treatment regimes. The concept of combining the passive nature of permeable barriers with a treatment processes that transforms or mineralizes the contaminant shows tremendous potential as a remedial design alternative.

The utility of biological processes for the remediation of contaminated groundwater has been realized. However, the additional regulatory burden associated with the injection of chemicals requisite for *in-situ* biological treatment detracted from the attractiveness of full-scale treatment systems. In the development of a biological permeable barrier for PCP contaminated groundwater, foresight was given to requite components of system operation. While many electron donors may support the anaerobic reductive dechlorination of PCP, **process development** of the biological treatment mechanisms focussed on the application and evaluation of a novel electron donor, imitation vanilla flavoring. The individual components of the electron donor mixture were GRAS by the FDA. Therefore, it was envisioned that the injection of chemicals GRAS to an aquifer system may have wider acceptance among the regulatory community and adjacent property owners. Laboratory serum bottle assays indicated imitation vanilla flavoring was an effective electron donor for anaerobic PCP reductive dechlorination. When supplied as the electron donor, imitation vanilla flavoring catalyzed PCP reductive dechlorination at all chlorine substituted positions. Furthermore, the presence of 3,4,5-TCP did not affect continued chlorophenol removal in the experimental system. The demonstrated success of imitation vanilla flavoring as an electron donor in biological groundwater remediation system was promising; it's physical, chemical and regulatory properties were ideal for use in a field scale application.

Technology development proceeded in unison with the identification of a suitable electron donor for the treatment process. A groundwater bioremediation system for *in-situ* PCP degradation was designed and constructed using a permeable barrier concept. In a PCP contaminated aquifer at the L.D. McFarland Facility in Eugene, Oregon a large diameter well was constructed. The well functioned as a cost effective means for passive groundwater interception. A cylindrical reactor was designed to fit within the casing. The unit was compartmentalized with porous vertical partitions, which, created three zones for biological treatment process and three zones for nutrient addition and mixing. The reactor assembly was then installed within the casing and suspended at a depth, which corresponded to the screened interval of the well. Biologically active zones were packed with municipal wastewater inoculated ceramic saddles. Imitation vanilla flavoring was supplied to the unit as an electron donor, mixing was accomplished through the addition of nitrogen and oxygen gas. The reactor was monitored with a custom designed pneumatic sampling system. Environmental conditions were measured and automatically logged in two positions in the treatment unit using recirculating flow cells. Development, design, construction and validation of the treatment system and its ancillary components was an arduous task.

Process and technology development was ultimately combined and culminated in the **pilot demonstration** which, was conducted at an active chemical wood treating facility. At pilot scale, the *in-situ* biodegradation of PCP contaminated groundwater was evaluated under several environmental conditions. In the presence of the inoculated permeable barrier, aqueous phase PCP was not degraded. Nor was PCP removed under oxidizing conditions in the presence of cells and imitation vanilla flavoring. Under anaerobic conditions however, PCP degradation was observed in the presence of imitation vanilla flavoring and cells. Chemical speciation of PCP degradation products indicated reductive dechlorination was the primary mechanism of removal. Degradation of PCP *in-situ* was complete in the pilot demonstration at the L.D. McFarland facility. Results from the pilot demonstration study indicate that biological permeable barriers are an effective tool for the remediation of contaminated groundwater. Following the successful demonstration of the biological permeable barrier, two field and laboratory companion studies were conducted.

In laboratory serum bottles and in the field pilot demonstration reactor the effect of electron donor concentrations on PCP reductive dechlorination was evaluated in companion study No. 1. While it seems logical to believe that increasing supplemental electron donor concentrations could result in higher transformation rates of PCP; results from companion study No. 1 proved otherwise. Results from the field and laboratory systems were complementary; when supplied 10 or 100 mg/L supplemental COD, there was no appreciable difference in the rate of PCP degradation. In laboratory and field systems evaluated, suspension of electron donor supply resulted in a decrease in the extent of PCP transformation. The performance of the *in-situ* permeable barrier was clearly compromised in the absence of donor addition. The result of donor termination was a corresponding increase in reactor system PCP concentrations. Operation of the pilot scale reactor in the absence of imitation vanilla flavoring was supported by observations made in the laboratory serum bottles; PCP transformation was possible without the supply of an external electron donor. Degradation of PCP in the laboratory proceeded by the sequential reduction of PCP's *ortho* chlorine atoms to yield 3,4,5-TCP, which accumulated. In contrast, operation of the biological permeable barrier in the field resulted in the complete removal of PCP; no accumulation of intermediate degradation products was observed.

Anaerobic processes are an effective mechanism for the biological treatment of highly chlorinated organic compounds. In the deployment of an anaerobic strategy for *in-situ* groundwater treatment, interference from competing biological processes exists. Companion study No. 2 was conducted to evaluate the effect of sulfate on PCP reductive dechlorination under laboratory and field conditions. Laboratory and field experiments revealed that sulfate was not inhibitory to PCP reductive dechlorination. However, laboratory PCP transformation rates in the presence of sulfate were noticeably slower in comparable systems that lacked sulfate. Sulfate addition to the pilot scale reactor had no apparent effect on the extent of PCP degradation. Complete PCP removal occurred in the system irregardless of sulfate addition. The laboratory degradation pathway of PCP in the presence or absence of sulfate was identical.

PCP reductive dechlorination followed sequential *ortho* chlorine cleavages to yield 3,4,5-TCP, which accumulated in laboratory serum bottles. In the field, PCP was fully degraded; no metabolic products of reductive dechlorination were observed. Overall, the results of companion study No. 2 suggest that anaerobic processes could be successfully implemented for the biological remediation of groundwater impacted by sulfate and chloroaromatic compounds.

Observation of metabolite accumulation in the serum bottles evaluated in companion studies No. 1 and No. 2 initiated a laboratory study investigate the effect of hydrogen on the reductive dechlorination of 3,4,5-TCP. In the presence of exogenous hydrogen, 3,4,5-TCP was degraded by reductive dechlorination. The results indicate that an inorganic electron donor, hydrogen can support the needs of an anaerobic consortium capable of reductive dechlorination. Production and accumulation of 3,4,5-TCP from the anaerobic reductive dechlorination of PCP is a potential pitfall in the application of a biological remediation system for impacted groundwater. Increased toxicity, solubility and mobility associated with 3,4,5-TCP accumulation detract from the convenience of *in-situ* biological treatment strategies. The factors contributing to the accumulation of intermediate metabolites from the reductive dechlorination of PCP are truly unknown. Therefore, in the application of biological treatment for PCP contaminated media, the potential for product accumulation always exists. The stimulation of 3,4,5-TCP reductive dechlorination through hydrogen addition illustrates an effective process modification for biological systems where metabolite accumulation is problematic.

Research at the laboratory, bench, pilot, and field scale provides countless examples of the transformation and degradation capacity of biologically mediated systems. Laboratory research has shown what contaminants are amenable to biological treatment while, pilot and field scale projects have demonstrated the most successful application methods. There is an inherent link between the long-term success of the field scale systems and fundamental treatment mechanisms derived under the confined and controlled laboratory environment. The link between the laboratory and field arenas allows an iterative approach to determine the environmental conditions in which a treatment system will adequately transform target components or ultimately fail. Beyond the scientific aspects, combined laboratory and field based studies allow for economic evaluations and optimization of a particular treatment technology. The optimization and economics of a treatment system are derived from the points in which the system can no longer operate within the ranges of desired efficiency. Scientifically, these points are defined by system failure resulting from the perturbation of components critical to fundamental unit operations. Continued research incorporating companion laboratory and field studies will help delineate the boundaries in which the aspects of *in-situ* biological treatment, engineering and economics are combined to develop cost effective solutions for groundwater remediation.

RESEARCH SUMMARY

In fulfillment of the overall program goal, research and development of the treatment system were distributed among three areas of focus: Process Development, Technology Development and the Pilot Demonstration. Specific detailed conclusions of the individual project components are listed below and summarized by chapter.

Chapter 2 Evaluation of Imitation Vanilla Flavoring to Support the Reductive Dechlorination of Pentachlorophenol

- Imitation vanilla flavoring was an effective electron donor for anaerobic PCP reductive dechlorination.
- When supplied as the electron donor, imitation vanilla flavoring was consumed in the biological transformation of PCP.
- PCP reductive dechlorination was catalyzed at all chlorine substituted positions and the presence of 3,4,5-TCP did not affect the continued chlorophenol removal in the experimental system.
- Transformation of PCP was rapid; 99% of the initial PCP mass was transformed in less than 85 hours.
- Overall, the results of this study indicate that imitation vanilla flavoring would be an effective electron donor for use in an in-situ biological treatment regime for PCP contaminated groundwater.

Chapter 3 Pilot Scale Demonstration of a Permeable Barrier Technology for the *in-situ* Bioremediation of Pentachlorophenol-Contaminated Ground Water

A bioremediation system for the in-situ degradation of pentachlorophenol was designed, developed and constructed from a conceptual treatment process. The system was based upon a permeable barrier concept and used a large diameter well for the passive interception of PCP contaminated groundwater.

- The site geology, nature of contamination, and thorough hydraulic control in conjunction with an enthusiastic site owner proved the L.D. McFarland Site in Eugene Oregon was and ideal location for the pilot demonstration. A good working relationship between the site owner and regulatory agency, the Oregon Department of Environmental quality was paramount to the success of the project.
- Nine months of data collection at the demonstration site indicated that PCP transformation by natural attenuation was negligible. Baseline data collection was one of the most important means to ascertain the contribution of the permeable barrier reactor for groundwater remediation.
- Baseline evaluation over a nine month period reveled a direct correlation between static water elevation and PCP loading rates in the permeable barrier reactor. Increases in water elevation associated with seasonal rains resulted in an increase in PCP influent to the treatment system.
- Conservative tracer studies indicated the hydraulic residence time in the reactor system was roughly 24 hours. The results of tracer studies conducted shortly after inoculation and

approximately one year later were complementary. These results suggest that the hydraulic residence time was unaffected by the growth of a biological population.

A removable, permeable barrier reactor and ancillary support systems were designed and constructed for the demonstration of an *in-situ* treatment system for the bioremediation of PCP contaminated groundwater.

- A cylindrical reactor body was designed and constructed to fit within the casing of a 24" diameter well previously installed at the demonstration site. Vertical partitions installed in the reactor assembly created three distinct biological treatment zones. Each treatment zone was preceded by a nutrient injection and mixing area. Spacing of the treatment zones was based on PCP degradation studies conducted in one-dimensional column studies.

- The reactor partitions, lifting mechanisms and static support systems were structurally designed to allow the use of a dense inexpensive porous media (e.g. pea gravel) in the biological treatment zones. Although ceramic saddles were used in the pilot demonstration, use of pea gravel as a medium for biological growth would offer tremendous cost savings in a field scale implementation.

- To weather the reduced, corrosive environment expected in the treatment well, the reactor assembly and fasteners used in fabrication were constructed of stainless steel. Teflon was selected for process piping and sample collection lines for its durability and low chemical reactivity.

- A gas lift mixing system for the addition of electron donor and acceptor pairs was designed and installed in the permeable barrier reactor.

- Under simulated field conditions, the operation of the reactor mixing and nutrient injection system was evaluated in the laboratory in a large water tank. Nutrient mixing and distribution was evaluated using dyes. Operating conditions were adjusted to provide the desired level of mixing. Mixing gas flow rates developed in the laboratory were initially used in field operation of the reactor system.

- A pneumatic sampling system was designed and installed in the reactor permeable barrier reactor. The Prior to use in the reactor, a prototype sampler was used for the collection of baseline groundwater samples. Validation of the pneumatic sampling systems was made by comparison of analytical groundwater results between conventional and prototype sample collection methods.

- A real time data collection system was installed to monitor environmental conditions present within the permeable barrier reactor assembly (e.g. pH, oxidation/reduction potential, conductivity, etc.). Data collected from this system was of particular value for assessing the overall microbial conditions present in the reactor system.

- Careful planning in the design and development stages allowed for the successful demonstration and operation of the permeable barrier reactors ancillary support and sampling systems

In-situ bioremediation of PCP contaminated groundwater was demonstrated at the pilot scale in a biological permeable barrier reactor installed at L.D. McFarland Facility in Eugene, Oregon. Chemical speciation of PCP degradation products indicated reductive dechlorination was the primary mechanism of removal.

- Based upon the chemical speciation of chlorophenols present in site groundwater, There was no evidence to suggest natural attenuation mechanisms for PCP. In the absence of electron donor supply, similar observations in reactor operation were made following inoculation.

- In the presence of the inoculated permeable barrier, aqueous phase PCP was not degraded. Nor was PCP removed under oxidizing conditions in the presence of cells and imitation vanilla flavoring. However under anaerobic conditions, PCP degradation was observed in the presence of imitation vanilla flavoring and cells.

- Environmental conditions measured in the treatment zones indicated that PCP biotransformation occurred under anaerobic conditions. Degradation of PCP in-situ was complete in the pilot scale demonstration at the L.D. McFarland facility. There was no evidence to suggest that aerobic mechanisms of PCP removal. Sequential anaerobic/aerobic operation of treatment zones in the reactor was a formidable task. Under the operation parameters and conditions evaluated, the establishment of treatment by sequential environments was not possible.

Chapter 4 Field and Laboratory Comparisons of Substrate Requirements for the Bioremediation of Pentachlorophenol-Contaminated Ground Water

- The reductive dechlorination of PCP was observed under field and laboratory conditions in the presence and absence of an exogenous electron donor, imitation vanilla flavoring. Results from the companion study were complementary. With the exception of systems lacking an exogenous donor, the rate of PCP transformation was independent of electron donor concentration. At all electron donor concentrations evaluated, PCP was transformed at nearly identical rates. In systems lacking electron donor addition, PCP transformation was observed. However, in comparison to systems where electron donor was added, a noticeable decrease in the extent of PCP transformation occurred.

- PCP was degraded in laboratory serum bottles and in the biological permeable barrier by the process of reductive dechlorination. Degradation of PCP in the laboratory proceeded by the sequential reduction of PCP's ortho chlorine atoms to yield 3,4,5-TCP, which accumulated. In systems supplied 50 and 100 mg COD/L, there was evidence to support further transformation of 3,4,5-TCP to 3,5 DCP. Operation of the biological permeable barrier in the field resulted in the complete removal of PCP. No accumulation of intermediate degradation products was observed.

- Pilot and laboratory degradation experiments were complementary. Like the serum bottle evaluated, PCP transformation was also observed in the pilot scale reactor in the absence of an external electron donor. Based upon the excellent performance of the serum bottle and in-situ reactor evaluated without supplemental COD, the contribution of alternate electron donors for PCP reductive dechlorination seems likely. The source and nature of the alternate electron donor was not determined.

- While the electron donor concentrations were not truly optimized, study results showed that PCP degradation was identical when supplied 10 mg COD/L or 100 mg COD/L of a supplemental electron donor. Suspension of electron donor supply to the reactor system had adverse effects on the pilot system performance and resulted in an increase in PCP concentrations in the in-situ permeable barrier. It is clear from both the laboratory and field data that the reductive dechlorination of PCP in the system investigated requires very little supplemental COD.

Chapter 5 The Effect of Sulfate on the Reductive Dechlorination of Pentachlorophenol: A Field and Laboratory Comparison

- Laboratory experiments revealed that sulfate was not inhibitory to PCP reductive dechlorination. However, laboratory PCP transformation rates in the presence of sulfate were noticeably slower in

comparable systems, which lacked sulfate. Sulfate addition to the pilot scale reactor had no apparent effect on the extent of PCP degradation. Complete PCP removal occurred in the system regardless of sulfate addition.

- The laboratory degradation pathway of PCP in the presence or absence of sulfate was identical. Laboratory degradation of PCP proceeded by reductive dechlorination and followed sequential ortho chlorine cleavages to yield 3,4,5-TCP. In the serum bottles evaluated, 3,4,5-TCP accumulated. In the field, PCP was fully degraded; no metabolic products of reductive dechlorination were observed.

- Overall, results of this study suggest that anaerobic processes could be successfully implemented for the biological remediation of groundwater impacted by sulfate and chloroaromatic compounds. Comparable behavior in system operation between field and laboratory experiments illustrates the utility of companion studies for the optimization of in-situ remediation systems.

Chapter 6 The Effects of Hydrogen on the Reductive Dechlorination of 3,4,5-Trichlorophenol

- In the presence of exogenous hydrogen, 3,4,5-TCP was degraded by reductive dechlorination. These results indicate that an inorganic electron donor, hydrogen can support the needs of an anaerobic consortium capable of reductive dechlorination

- The observed reductive dechlorination of 3,4,5-TCP following the addition of hydrogen to an experimental system previously shown to accumulate 3,4,5-TCP suggests that the serum bottles were hydrogen limited. It appears that hydrogen addition may stimulate 3,4,5-TCP degradation in systems where metabolite accumulation occurs.

- The minimum hydrogen concentration for 3,4,5-TCP reductive dechlorination appears to fall between 0.0015 and 0.005 atm. Further studies are required to closer estimate the minimum hydrogen required by this culture.

- Observation of a sole metabolic product 3,5-DCP, suggests that reductive dechlorination of 3,4,5-TCP occurred exclusively in the para position. A mass balance on chlorophenols in the system, and the lack of stoichiometry observed in 3,4,5-TCP transformation suggests that 3,5-DCP was further degraded.

- Production and accumulation of 3,4,5-TCP from the anaerobic reductive dechlorination of PCP is a potential pitfall in the application of a biological remediation system for impacted groundwater. Results of this study suggest new methods for biological systems where metabolite accumulation is problematic. The stimulation of 3,4,5-TCP reductive dechlorination through hydrogen addition has great utility in the development of field based treatment systems.

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APPENDICES

APPENDIX A LIST OF FILENAMES

WORD FILES

Dissertation.doc	Complete dissertation of Jason David Cole

ACCESS FILES

Cell97.mdb	Master record for all flow cell data collected during the field demonstration
MCFC.mdb	Master record for all other field data collected during the demonstration

EXCEL FILES

BT_No1.xls	Imitation vanilla flavoring bottle test
BT_No2.xls	Evaluation of electron donor concentration
BT_No3.xls	Effects of competitive electron acceptors on PCP degradation
Batch_No1.xls	Effects of hydrogen partial pressure on 3,4,5-TCP degradation
COD_Data.xls	Reduced COD and anion/cation data from McFC.mdb
COD_Field.xls	
Flowcells.xls	Reduced flowcell data from Cell.mdb used for plot generation only
Mixing_D.xls	Reduced chlorophenol data from MCFC.mdb used for plot generation only
Tracer_No6.xls	Field data collected from fall 1997 tracer study
Tracer_No7.xls	Field data collected from fall 1998 tracer study

AUTOCAD RELEASE 14

Reactor.dwg	Design, construction and schematic drawings of the permeable barrier reactor
Oreactor.dwg	Orthogonal view drawing of the permeable barrier reactor
Mixing.dwg	Reactor mixing system schematic and process flow diagram
Cellpipe.dwg	Reactor flow cell sample system schematic and process flow diagram
Flowcell.dwg	Design & construction drawings for reactor flow cells
Wellsup.dwg	Design & construction drawings for reactor support components
Tower.dwg	Design & construction drawings for reactor lifting tower
Wells.dwg	Sketch plan of well location, tracer release and sample points

POWER POINT

Figure.ppt	PCP Degradation pathway drawings and organization charts
Defense.ppt	Complete oral defense presentation

ENDNOTE VERSION 2.0

Penta.enl	Complete endnote library of PCP research citations used in dissertation

MISCELLANEOUS

21x1.dat	Raw data from Campbell scientific data logger in comma delimited text format
Hach.txt	Raw data from Hach DR 890 colorimeter in comma delimited text format

APPENDIX B PERMEABLE BARRIE R REACTOR CONSTRUCTION

COMPONENT DESCRIPTION

The down-borehole reactor is a passive, in-situ, permeable biological reactor that allows the introduction of nutrients and other chemicals to a subsurface biological population. Equipped with sensors, nutrient delivery, and mixing systems, the reactor is installed in a large diameter well screened over an interval of the contaminated aquifer. Biodegradation of the aqueous phase organic compounds occurs over the length of the reactor in a combination of anaerobic and aerobic biological zones. The goal of this research is to demonstrate the applicability of a permeable barrier for the bioremediation of groundwater contaminated with pentachlorophenol (PCP). The ultimate goal is to develop this technology for the treatment of groundwater contaminated with complex waste mixtures.

A custom fabricated permeable barrier reactor was designed and constructed by the engineering service shop at Oregon State University. Many components used in the reactor were not commercially available. Therefore, custom fabrication was required. Specifications and construction drawings of the individual reactor components are summarized in Table B.1

Table B.1 Summary of reactor component construction plans

Drawing Description	Figure Number
Permeable Barrier reactor plan view and over all component layout.	Figure B.1
Orthogonal view of the reactor system reveling treatment and mixing zones	Figure B.2
Specifications and fabrication layout of reactor cover plate	Figure B.3
Specifications and fabrication layout of reactor side plates	Figure B.4
Construction detail drawing for fabrication of modular mixing zones	Figure B.5
Specifications and fabrication layout of reactor base plate	Figure B.6
Specifications and fabrication layout for reactor support and lifting bars	Figure B.7
Specifications and fabrication layout for reactor static support ring	Figure B.8
Specifications and fabrication layout for well support ring bushing plate	Figure B.9
Lifting tower construction plans-plan view	Figure B.10
Lifting tower construction plans-plan view	Figure B.11

The cylindrical unit is constructed of modular partitions and treatment cells. The reactor, shown in Figure B.1 is assembled to operate with three biologically active zones. Growth within these zones is supported on ceramic saddles that possess both high surface area and hydraulic conductivity. Each treatment zone is separated with open vertical partitions that serve as nutrient supply and mixing areas. Nutrient addition consists of continuous low flow injection of a highly concentrated aqueous feed solution supplied through a diffuser located at the base of mixing zone. Periodic agitation of the treatment zone influent is conducted by a gas lift mixing scheme. Inert gas or oxygen is used in the mixing regime depending on the desired environmental condition of the biological zone. All nutrient supply systems are isolated to allow for independent operation regardless of location within the reactor.

Essentially the reactor system is rectangular. From the plan view show in Figure B.1, the rectangular treatment zones with curved inlet and exit planes are visible. To minimize the effect of short-circuiting in the well casing, protect the nutrient, and sample systems from well casing abrasion, stainless steel sheets were fabricated and attached to the reactor. The plates result in the overall cylindrical appearance of the unit. With the cylindrical side panels removed, the internal configuration of the reactor is shown in Figure B.2. The unit is comprised of base plate, top plate and two side plates. The mixing zones in the reactor are of modular construction and are secured to the reactor side plates. In the well, the reactor is suspended from three stainless steel bars attached to the reactor base plate. Support bars placed at the front and rear of the unit are connected perpendicular to flow by stainless steel bridles. A pair of stainless steel cables transfers the reactor weight from the bridles to the surface. In static operation, the reactor is supported in the well by steel ring, which hangs on the top edge of the well casing. The support cables are attached to bearing plate that allows for rotation of the reactor inlet aperture. Installation and removal of the reactor in the well is accomplished with a collapsible derrick. The tower is set up over the well casing and equipped with an electric winch to ease in reactor movement. The unit is lifted and lowered on a single stainless steel cable that is connected by bridle to a lifting bar located in the center of the reactor base plate.

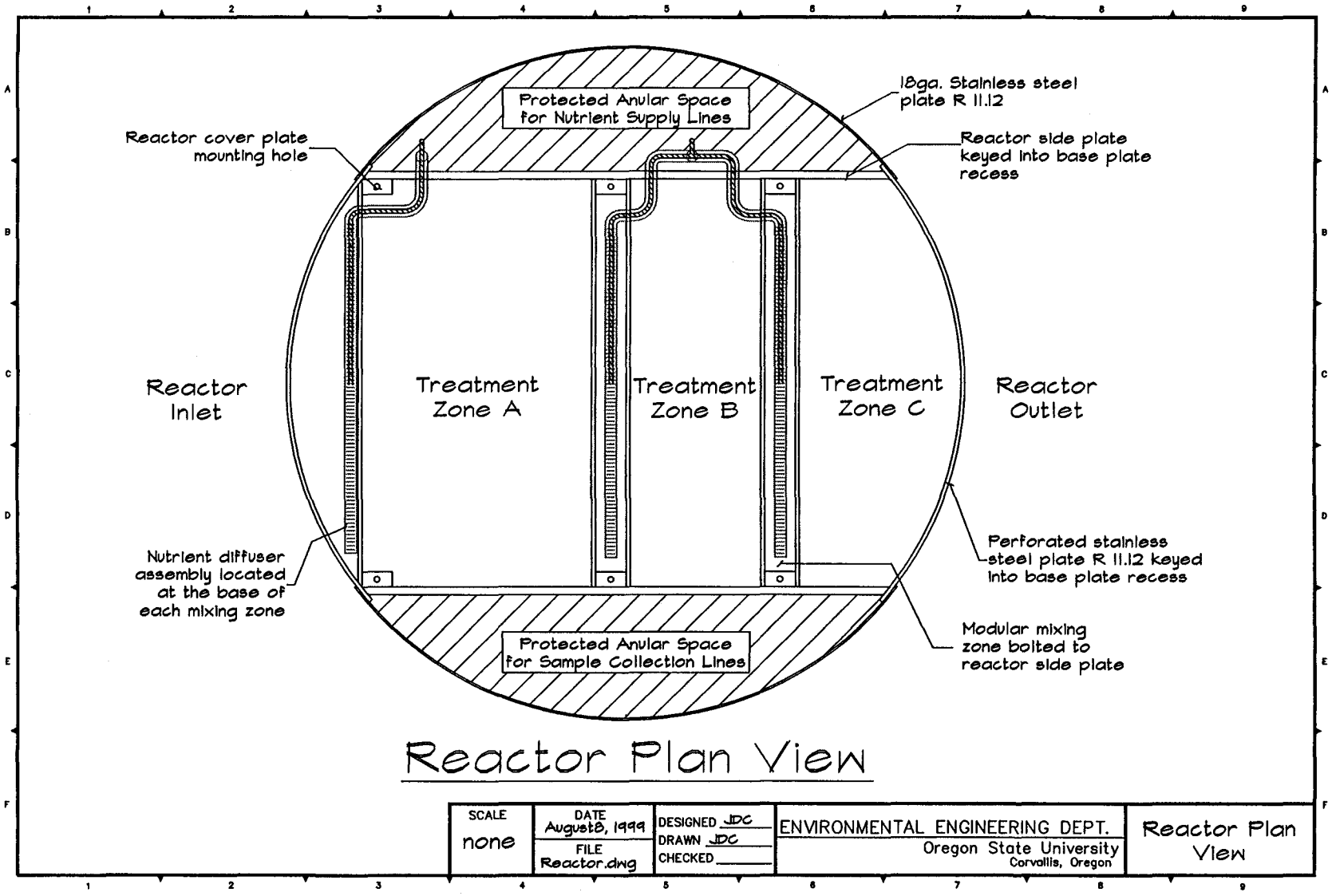

Figure B.1 Permeable barrier reactor plan view

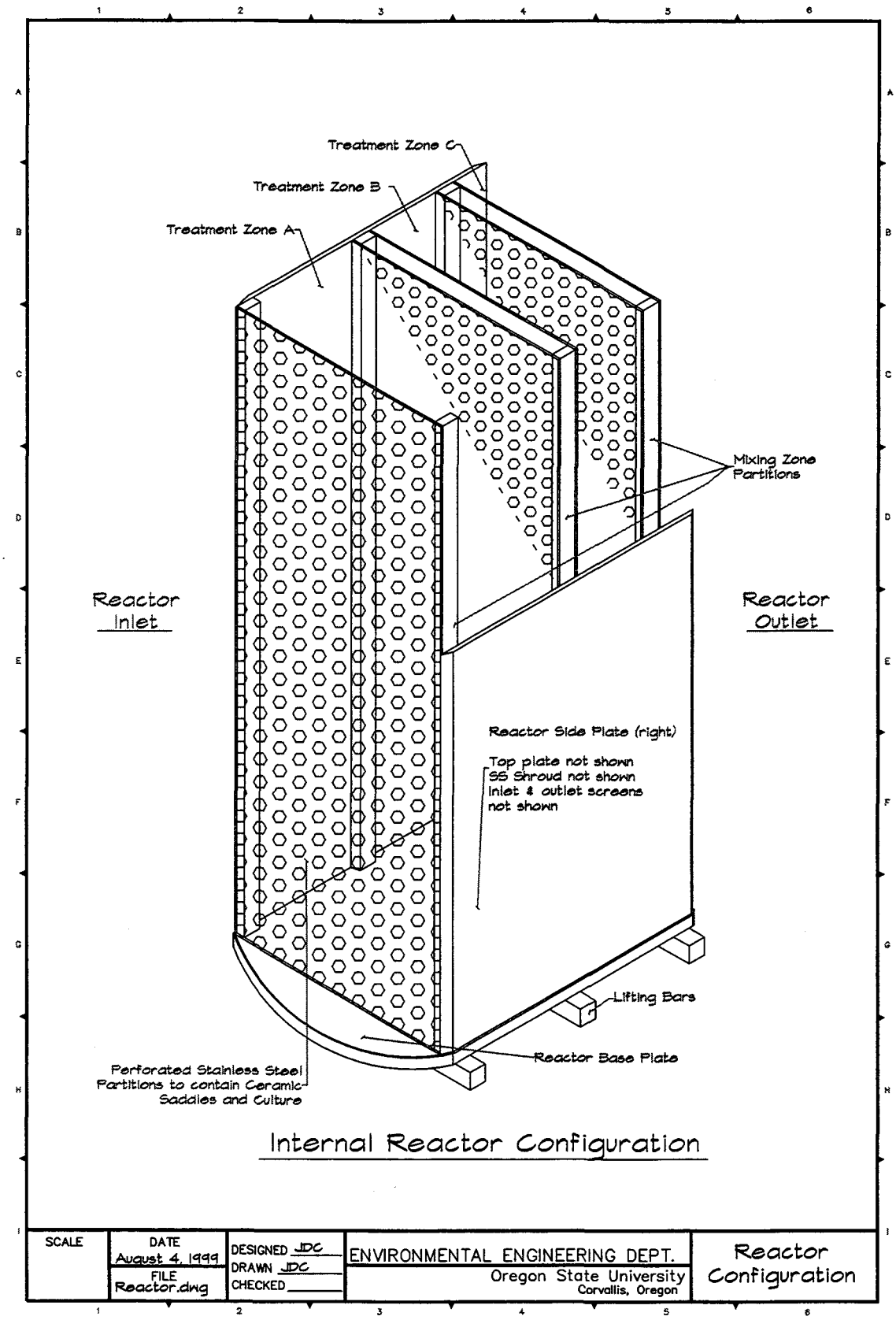

Figure B.2 Permeable barrier reactor internal configuration

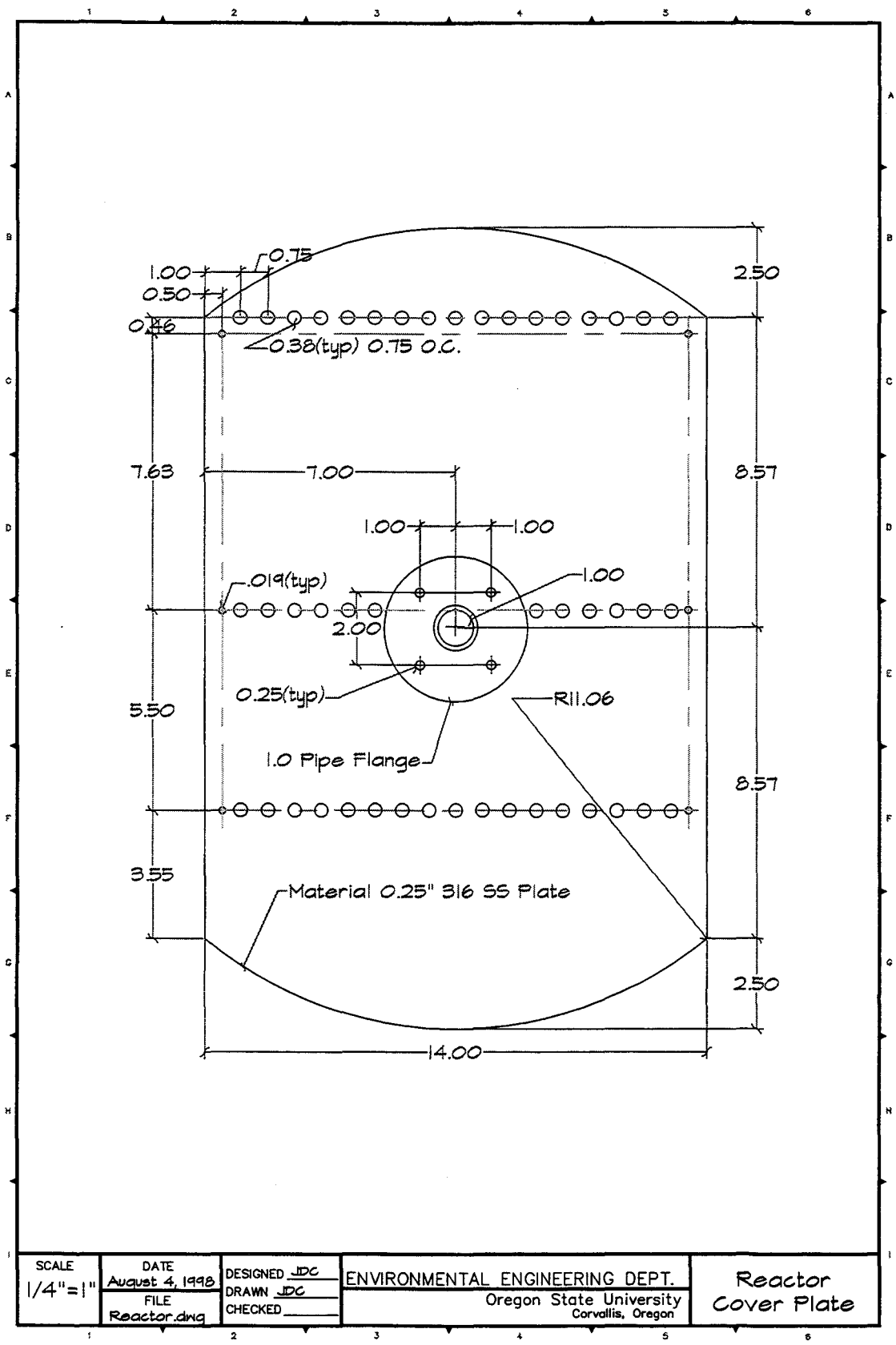

Figure B.3 Reactor cover plate

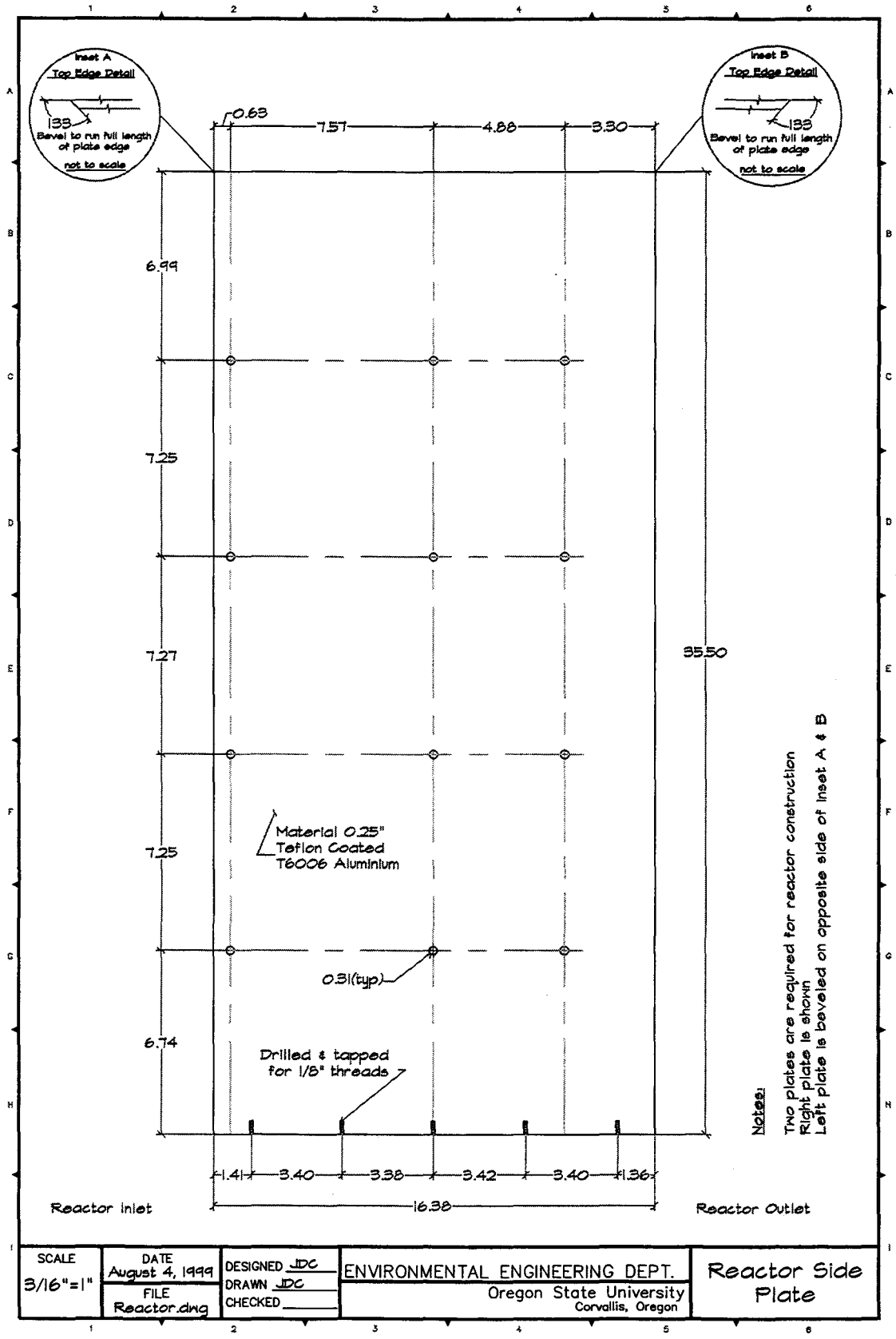

Figure B.4 Permeable barrier reactor side plate

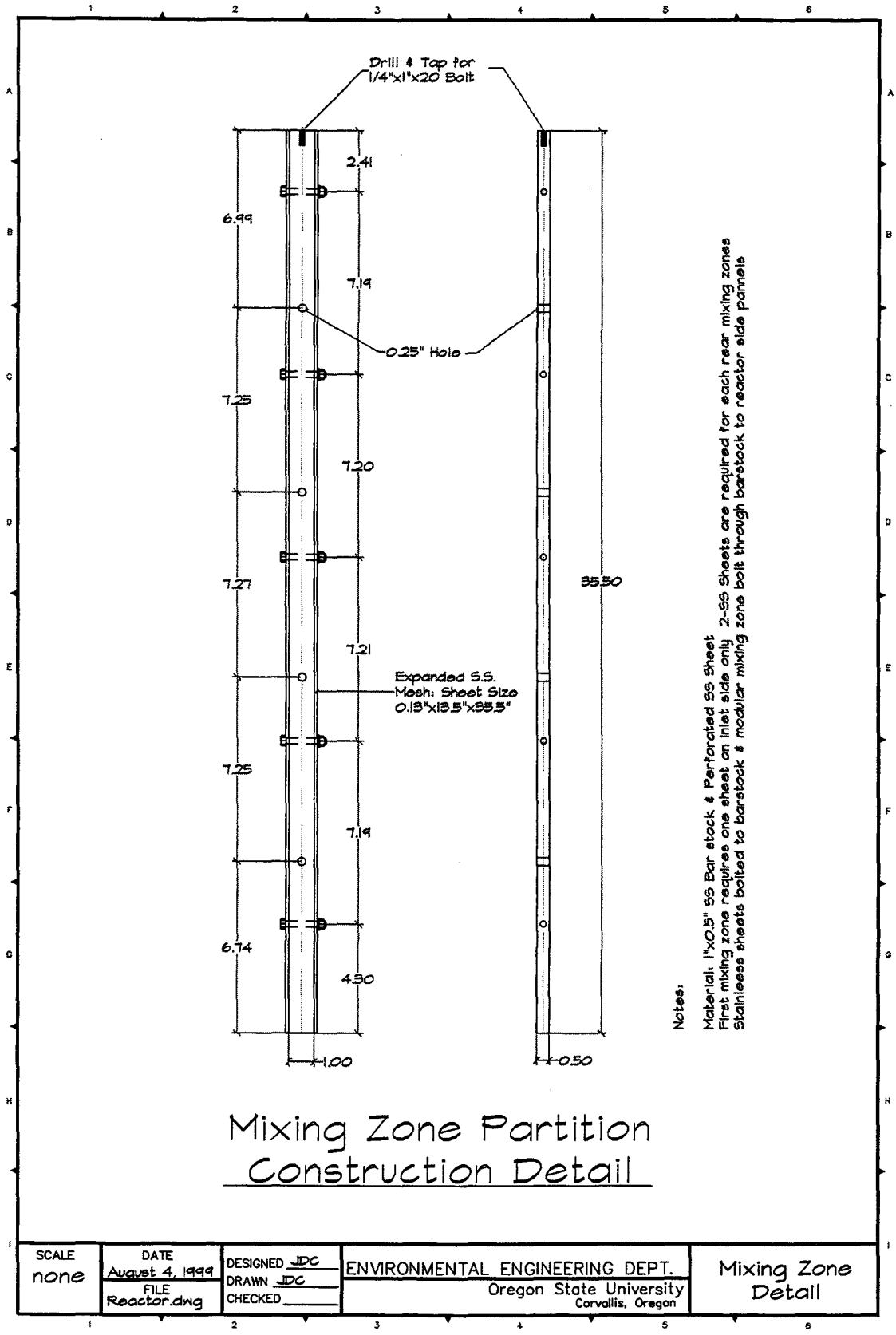

Figure B.5 Permeable barrier reactor mixing zone detail

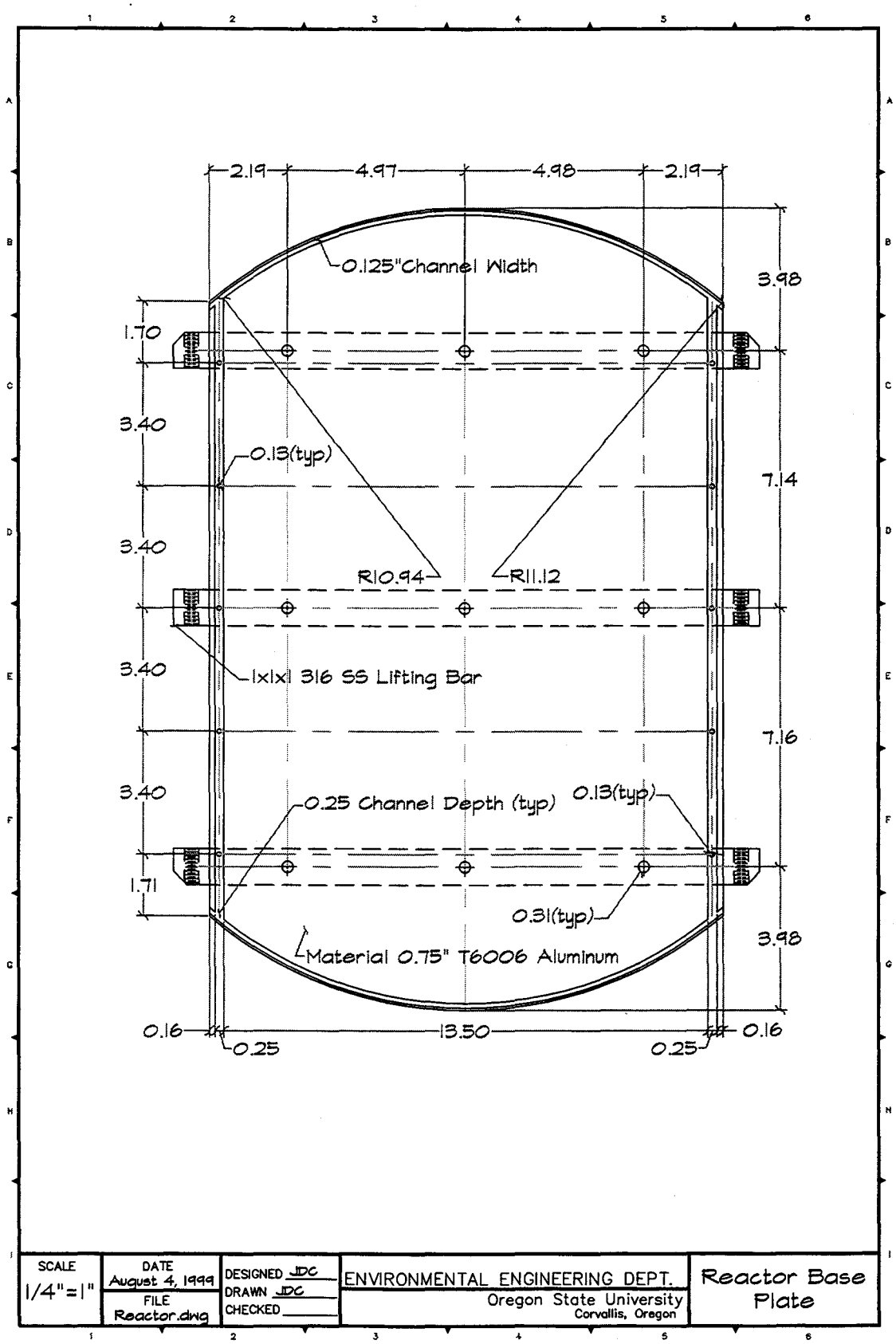

Figure B.6 Permeable barrier reactor base plate

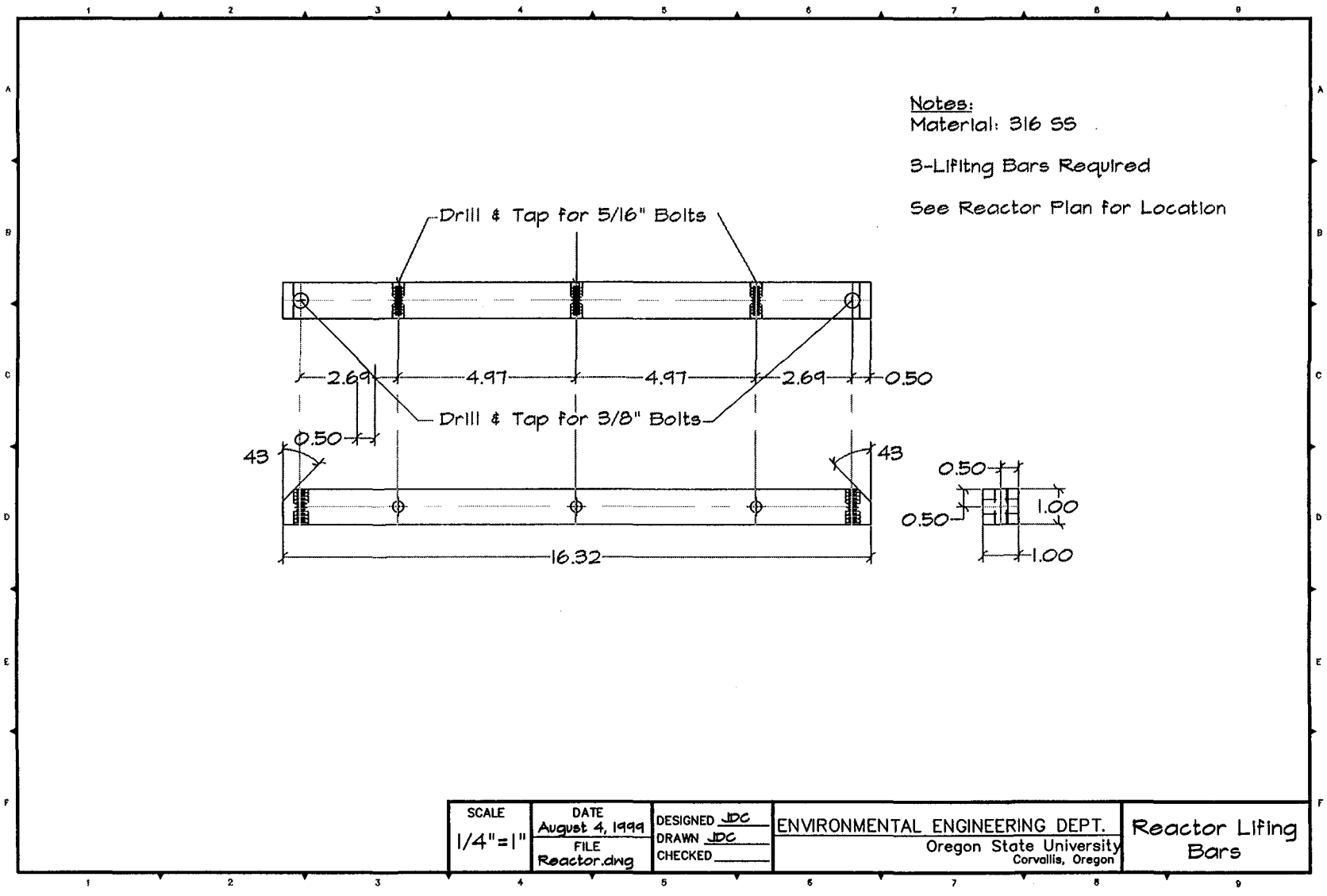

Figure B.7 Permeable barrier reactor lifting bars

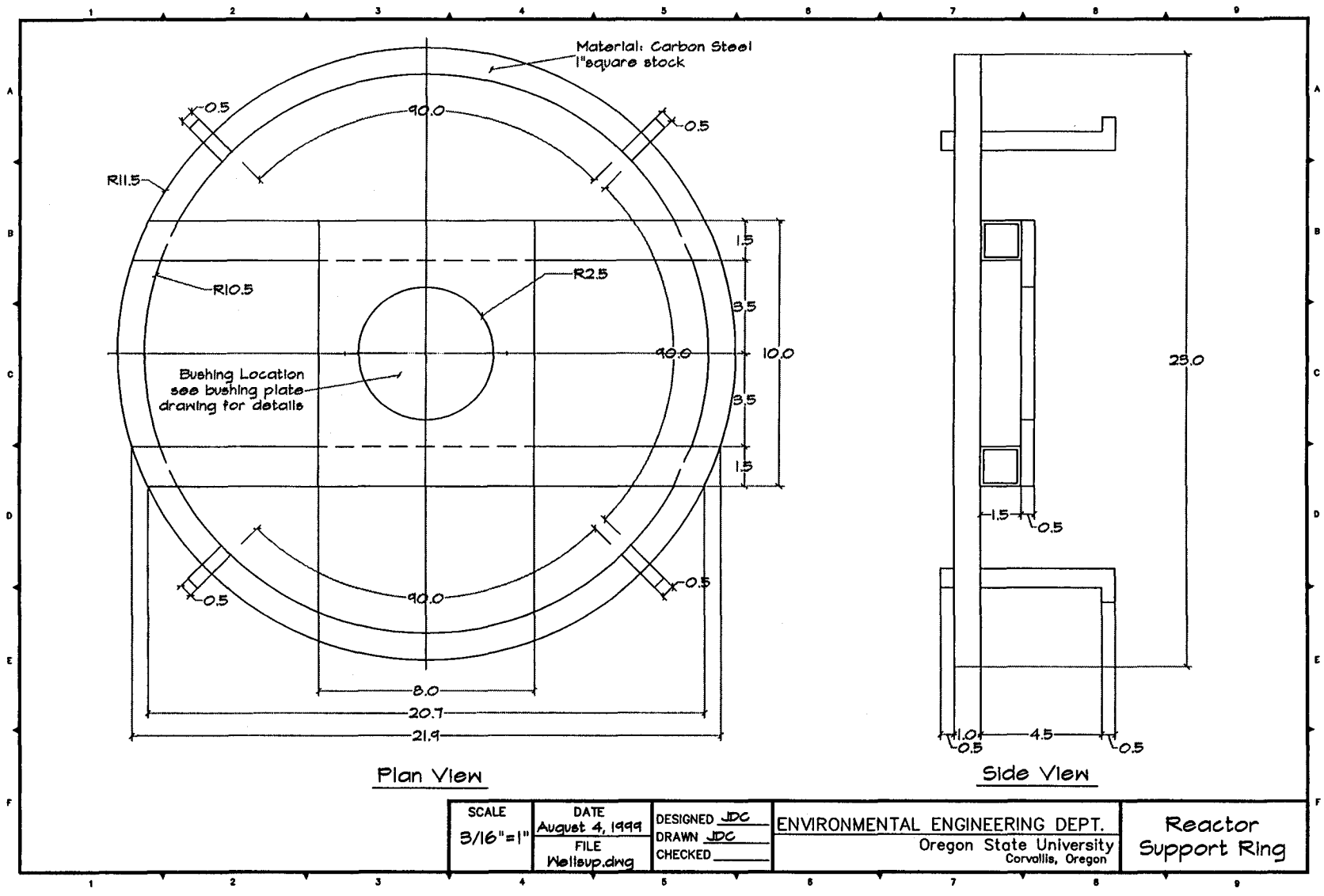

Figure B.8 Permeable barrier reactor well support ring

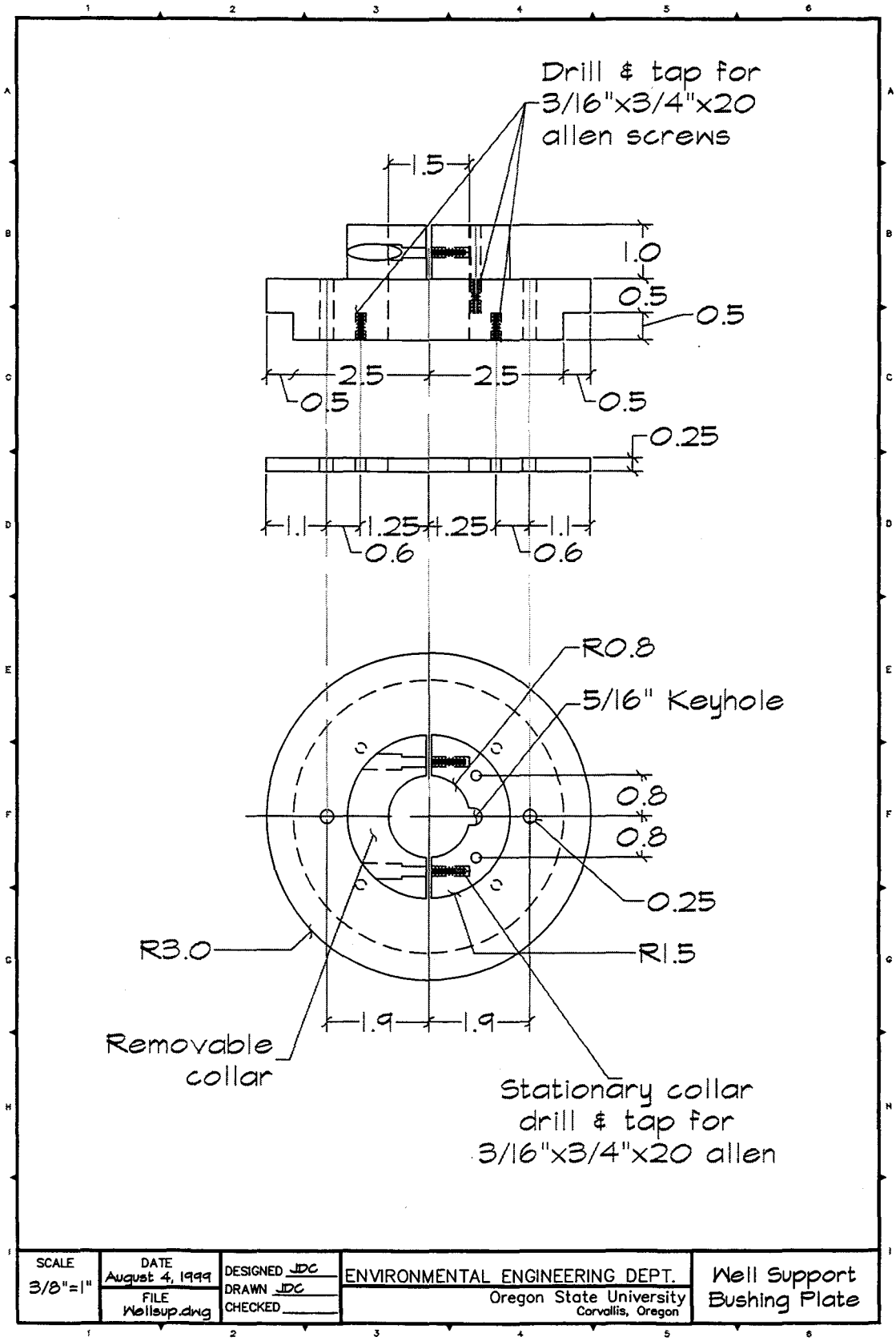

Figure B.9 Permeable barrier reactor well support bushing plate

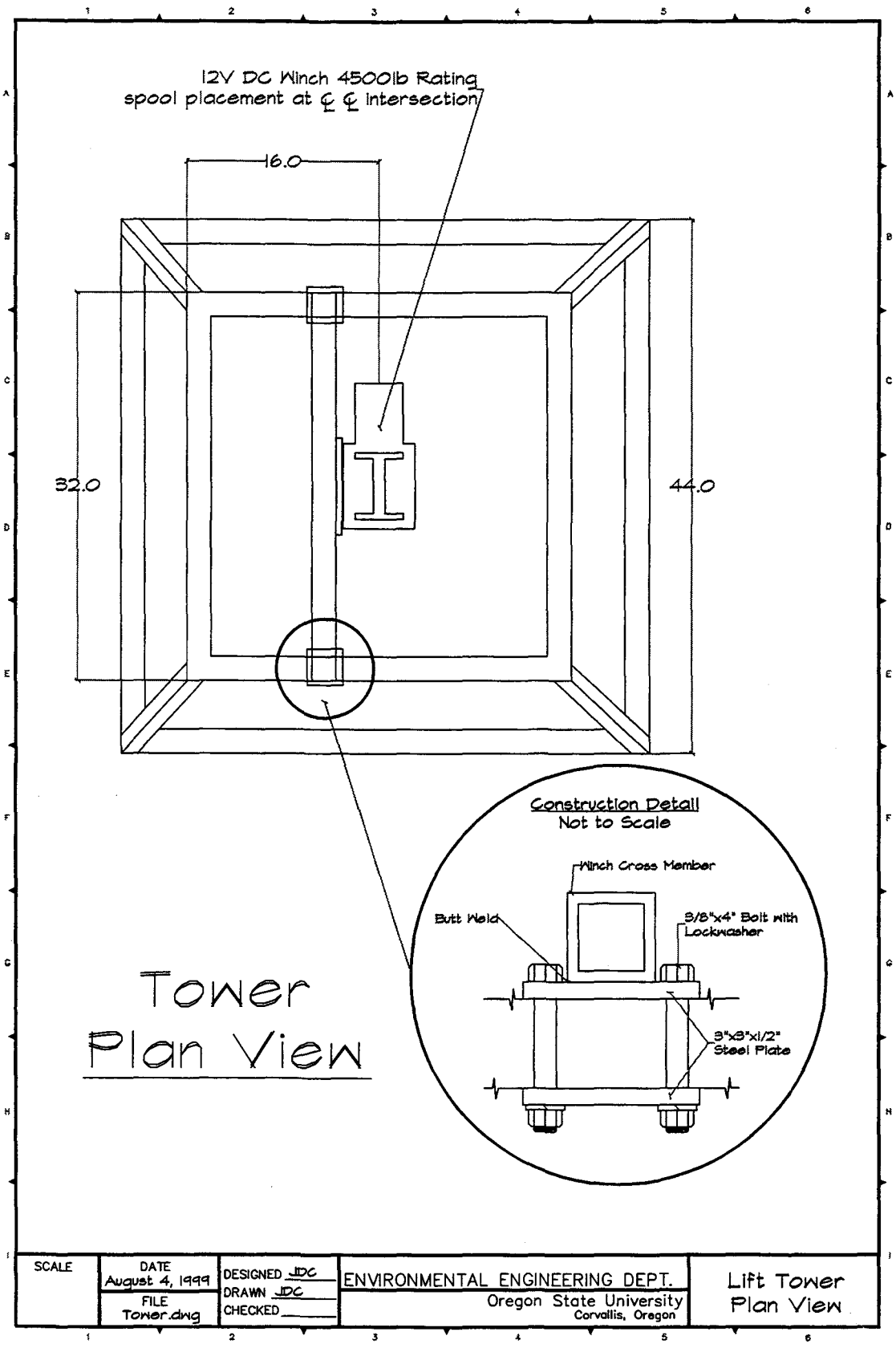

Figure B.10 Permeable barrier lifting tower plan view

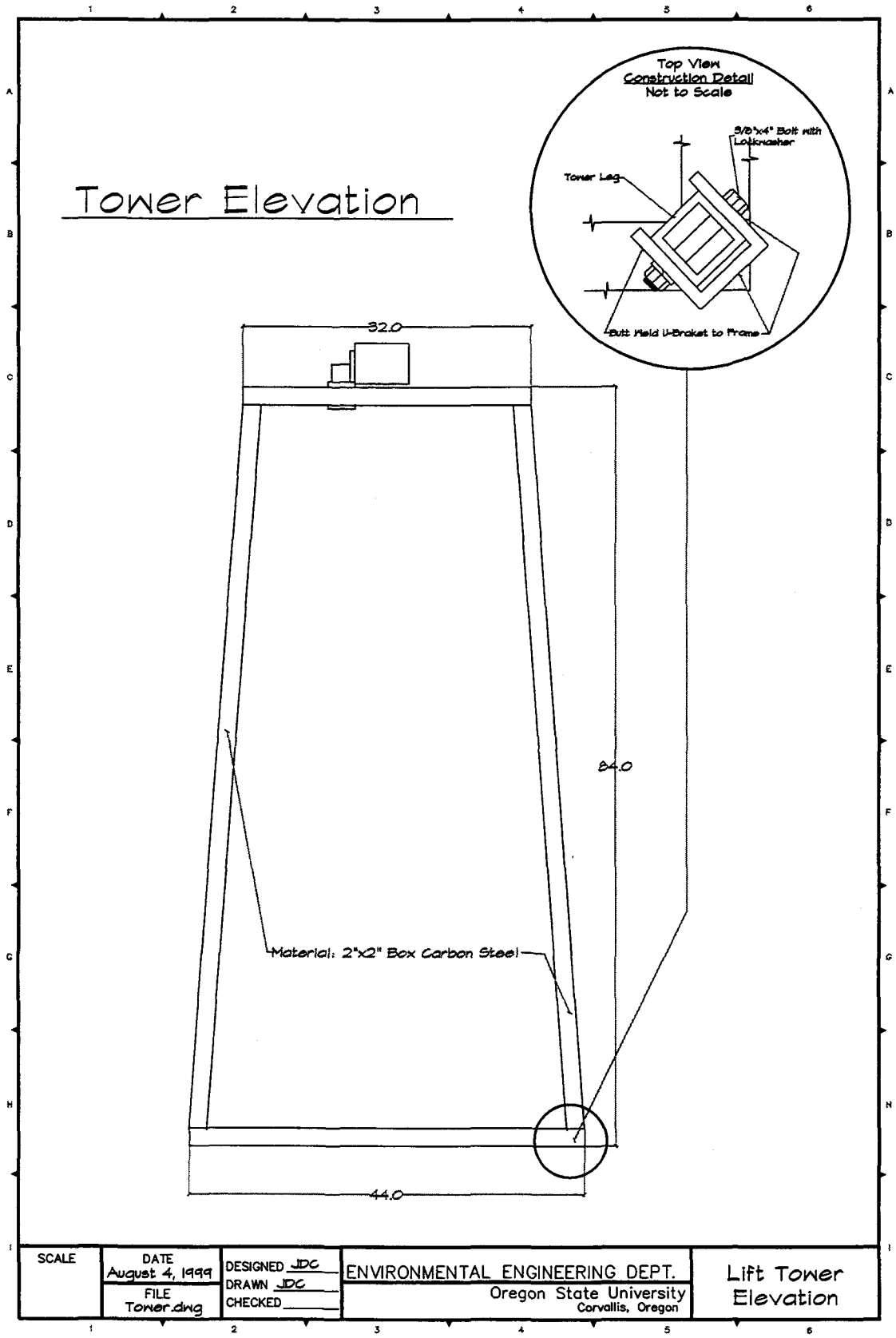

Figure B.11 Permeable barrier lifting tower elevation

APPENDIX C REACTOR NUTRIENT SUPPLY AND MIXING SYSTEM

COMPONENT DESCRIPTION

Mixing and nutrient supply systems for the permeable barrier reactor operated in unison. In the base of each mixing zone, nutrient supply lines joined a horizontal diffuser, creating a nested tube assembly. The diffuser was constructed of slotted stainless steel tubing and extended the complete width of the mixing zone. When supplied with a charge of compressed gas, nutrients and water were displaced from the diffuser assembly. Ejection of water and gas through the diffuser slots provided an opportunity for vigorous nutrient mixing and delivery. Vertical distribution of the mixture occurred by gas lift when, the buoyant gas bubbles rose upward through the open mixing zone. The diffuser slots were oriented toward the rigid screen boundary of the mixing and biologically active zone. The screen functioned as a baffle and helped to slow and break the upward flow of large gas bubbles from the diffuser assembly. A construction detail of the diffuser assembly is presented in Figure C.1. Orientation and overall placement of the diffuser unit is shown in Figure C.2.

Operation and control of the mixing and nutrient supply system in the first treatment zone was independent from the tandem operation of the second and third treatment zones. Electron donor was continuously pumped to the reactor mixing zones through 1/8" O.D. (3.2 mm) Teflon® tubing and two FMI QG-6 positive displacement pumps, Fluid Metering Inc. (Oyster Bay, NY). Two standard size gas cylinders and two-stage regulators were used to supply low pressure mixing gas to the diffuser assemblies. Two adjustable electric solenoid valves Cole-Parmer® (Vernon Hills, IL) controlled Mass flow of the mixing gases. Gas was supplied from the mass flow controllers to the reactor mixing zones through ¼" O.D. (6.4 mm) Teflon® tubing. The valve system used for mixing allowed control of both duration and frequency of activation. The nutrient injection and mixing system in the permeable barrier reactor is summarized in Figure c.2.

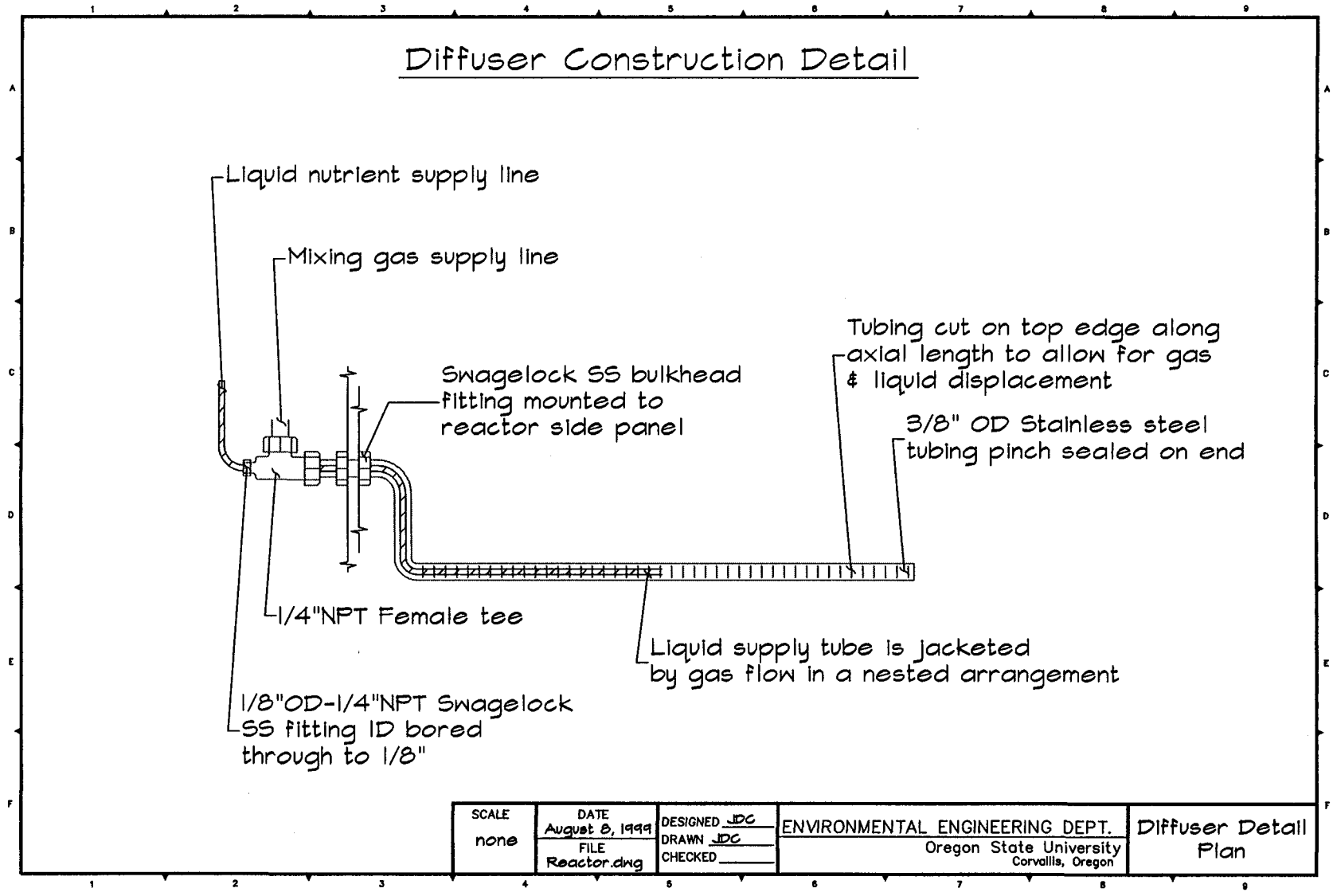

Figure C.1 Mixing zone diffuser construction detail

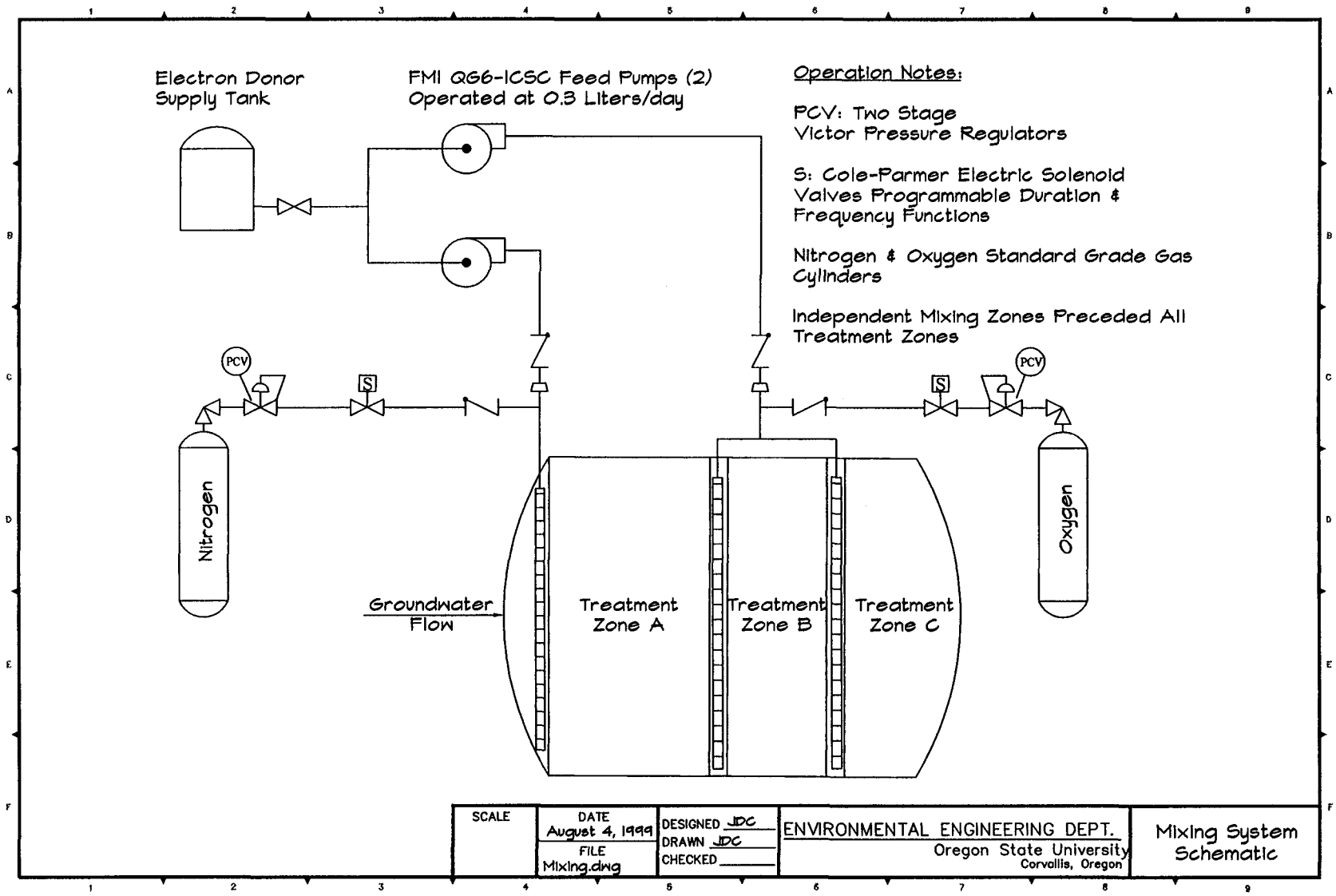

Figure C.2 Reactor nutrient supply & mixing system schematic

APPENDIX D MEASUREMENT OF R E A CTOR ENVIRONMENTAL CONDITIONS: FLOW CELLS

COMPONENT DESCRIPTION

Sensing the need to continuously monitor environmental conditions within the reactor's biological treatment zones, two continuous flow cells were constructed and installed on recirculating sample loops. A schematic process flow diagram of the flow cell monitoring system is shown in Figure D.1. Water was pumped from the center of the anaerobic and aerobic treatment zones by a dual channel Masterflex® peristaltic pump, Cole-Parmer® (Vernon Hills, IL). To minimize solids uptake, sample inlets were screened with No.40 stainless steel mesh, McMaster-Carr Co. (Los Angles, CA). Samples were collected with PEEK 1/8"O.D. (3.2 mm) tubing Alltech Associates, Inc. (Deerfield, IL) to minimize oxygen diffusion. Teflon® 1/8"O.D. (3.2 mm) tubing was used for the gravity return line. The flow cells were custom designed and constructed with acrylic plastic (Figure D.2). The finished internal volume of the flow cell measured 44 ml. Water was pumped from the two locations at 10 ml/minute to the base of each cell and flowed upward to the exit. The top of each cell was tapered to expedite the release of gas introduced by the pump. Low flow rates and equivalent mass removal and injection with the continuous loop design minimized preferential flow through the reactor. Both cells were completely mixed with magnetic plate assemblies and Teflon® coated stir bars. Spacers below the flow cells helped to minimize heat transfer from the stir plate.

The flow cells were designed to allow the use of three standard sized electrodes. Oxidation/reduction potential (E_H) and pH were measured real time in each cell using a pH combination glass body electrode Cole-Parmer® (Vernon Hills, IL) and a platinum E_H half cell, Analytical Sensors, Inc. (Concord, NH). The combination pH electrode served as a common reference (Ag/AgCl gel) for each cell. The probes were routinely cleaned and calibrated in accordance with the manufacturer specifications. A custom interface was designed to handle the electrode signals in each flow cell. Type T copper-Constantine thermocouples, Cole-Parmer® (Vernon Hills, IL), were used to monitor temperature differences between the groundwater system and the continuous flow cells. A Campbell Scientific 21X data logger (Logan, UT) was used for signal interpretation and data storage. The program written for the 21X data logger is shown in Appendix E. On regular intervals, data was manually transferred to a portable computer and interpreted.

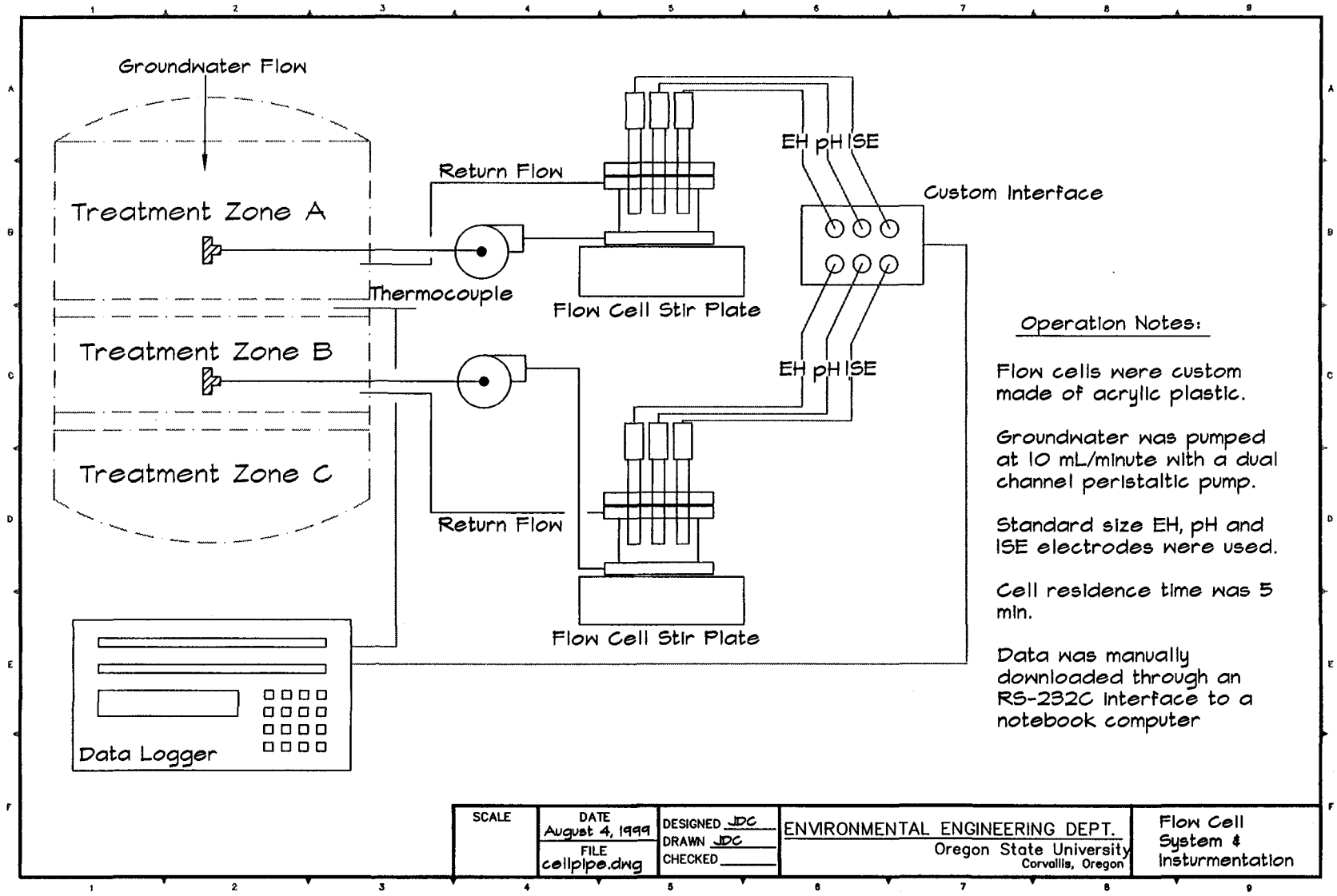

Figure D.1 Flow cell piping and instrumentation schematic

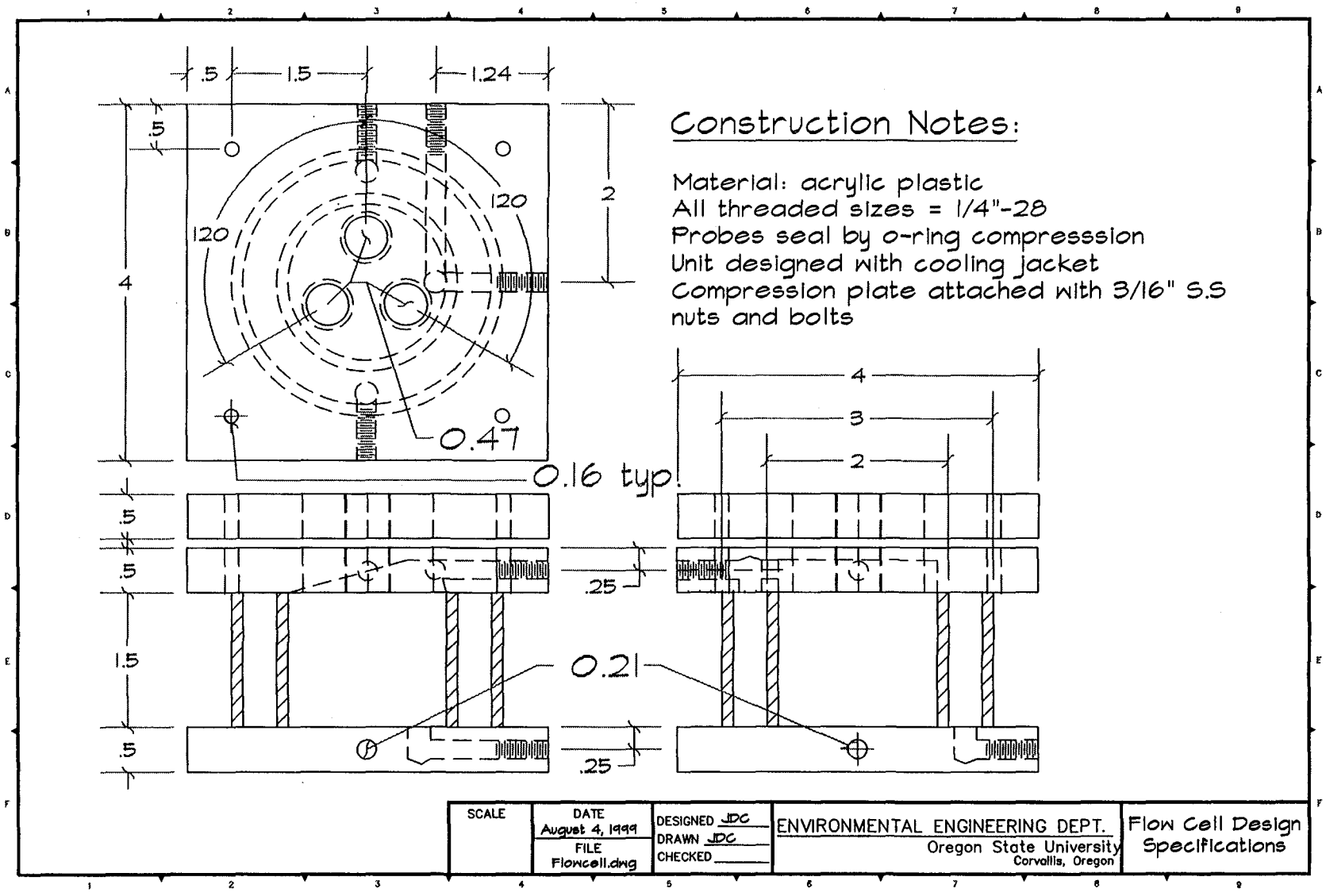

Figure D.2 Flow cell design specifications

APPENDIX E FLOW CELL DATA LO GGER OPERATION PROGRAM

COMPONENT DESCRIPTION

To collect data from continuous flow cells at the McFarland demonstration site, a Campbell Scientific 21X data logger was used. The following program was used to collect and process signal output from two thermocouples, two pH probes, two E_H probes and two ion specific electrodes. Channel assignments and names represent signal inputs described.

DATA LOGGER PROGRAM

```
*Table 1 Program
 01: 5       Execution Interval (seconds)

1: Internal Temperature (P17)
 1: 2       Loc [ 21xTemp   ]

2: Batt Voltage (P10)
 1: 1       Loc [ 21xBat    ]

3: Volt (Diff) (P2)
 1: 1       Reps
 2: 4       500 mV Slow Range
 3: 1       DIFF Channel
 4: 4       Loc [ EpH_A     ]
 5: 1.0     Mult
 6: -378.36 Offset
4: Z=X*F (P37)
 1: 4       X Loc [ EpH_A     ]
 2: -.0178  F
 3: 5       Z Loc [ pH_A      ]

5: Volt (Diff) (P2)
 1: 1       Reps
 2: 4       500 mV Slow Range
 3: 2       DIFF Channel
 4: 6       Loc [ Eh_A      ]
 5: 1.0     Mult
 6: 0.0     Offset

6: Volt (Diff) (P2)
 1: 1       Reps
 2: 4       500 mV Slow Range
 3: 3       DIFF Channel
 4: 7       Loc [ ISE_A     ]
 5: 1.0     Mult
 6: 0.0     Offset

7: Thermocouple Temp (DIFF) (P14)
 1: 1       Reps
 2: 12      15 mV Fast Range
 3: 4       DIFF Channel
 4: 1       Type T (Copper-Constantan)
 5: 2       Ref Temp Loc [ 21xTemp   ]
 6: 3       Loc [ Cell_Temp ]
 7: 1.0     Mult
 8: 0.0     Offset

8: Volt (Diff) (P2)
 1: 1       Reps
 2: 4       500 mV Slow Range
 3: 5       DIFF Channel
 4: 9       Loc [ EpH_B     ]
 5: 1.0     Mult
 6: -383.78 Offset
9: Z=X*F (P37)
 1: 9       X Loc [ EpH_B     ]
 2: -.0172  F
 3: 10      Z Loc [ pH_B      ]

10: Volt (Diff) (P2)
 1: 1       Reps
 2: 4       500 mV Slow Range
 3: 6       DIFF Channel
 4: 11      Loc [ Eh_B      ]
 5: 1.0     Mult
 6: 0.0     Offset
11: Volt (Diff) (P2)
 1: 1       Reps
 2: 4       500 mV Slow Range
 3: 7       DIFF Channel
 4: 12      Loc [ ISE_B     ]
 5: 1.0     Mult
 6: 0.0     Offset

12: Thermocouple Temp (DIFF) (P14)
 1: 1       Reps
 2: 12      15 mV Fast Range
 3: 8       DIFF Channel
```

```
 4: 1      Type T (Copper-Constantan)
 5: 2      Ref Temp Loc [ 21xTemp   ]
 6: 8      Loc [ Well_Temp ]
 7: 1.0    Mult
 8: 0.0    Offset

13: If time is (P92)
 1: 0      Minutes (Seconds --) into a
 2: 20     Interval (same units as above)
 3: 30     Then Do

14: Do (P86)
 1: 10     Set Output Flag High

15: Set Active Storage Area (P80)
 1: 1      Final Storage
 2: 1      Array ID

16: Real Time (P77)
 1: 110    Day,Hour/Minute (midnight = 0000)

17: Average (P71)
 1: 1      Reps
 2: 2      Loc [ 21xTemp   ]

18: Average (P71)
 1: 1      Reps
 2: 3      Loc [ Cell_Temp ]

19: Average (P71)
 1: 1      Reps
 2: 5      Loc [ pH_A      ]

20: Average (P71)
 1: 1      Reps
 2: 6      Loc [ Eh_A      ]
21: Average (P71)
 1: 1      Reps
 2: 7      Loc [ ISE_A     ]

22: Average (P71)
 1: 1      Reps
 2: 8      Loc [ Well_Temp ]

23: Average (P71)
 1: 1      Reps
 2: 10     Loc [ pH_B      ]

24: Average (P71)
 1: 1      Reps
 2: 11     Loc [ Eh_B      ]

25: Average (P71)
 1: 1      Reps
 2: 12     Loc [ ISE_B     ]

26: Sample (P70)
 1: 1      Reps
 2: 1      Loc [ 21xBat    ]

27: Do (P86)
 1: 20     Set Output Flag Low

28: End (P95)
*Table 2 Program
 02: 0.0000   Execution Interval (seconds)

*Table 3 Subroutines
End Program
```

PROGRAM REGISTRY

Final Storage Label File for: PH_EH21.CSI
Date: 7/8/1998
Time: 14:52:12

1 Output_Table 5.00 Sec
1 1 L
2 Day_RTM L
3 Hour_Minute_RTM L
4 21xTemp_AVG L
5 Cell_Temp_AVG L
6 pH_A_AVG L
7 Eh_A_AVG L
8 ISE_A_AVG L
9 Well_Temp_AVG L
10 pH_B_AVG L
11 Eh_B_AVG L
12 ISE_B_AVG L
13 21xBat L

Input Locations-
1 21xBat 1 1 1
2 21xTemp 1 3 1
3 Cell_Temp 1 1 1
4 EpH_A 1 1 1
5 pH_A 1 1 1
6 Eh_A 1 1 1
7 ISE_A 1 1 1
8 Well_Temp 1 1 1
9 EpH_B 1 1 1
10 pH_B 1 1 1
11 Eh_B 1 1 1
12 ISE_B 1 1 1
13 _____ 1 0 0
14 _____ 0 0 0
15 _____ 0 0 0
16 _____ 0 0 0
17 _____ 0 0 0
18 _____ 0 0 0
19 _____ 0 0 0
20 _____ 0 0 0
21 _____ 0 0 0
22 _____ 0 0 0
23 _____ 1 0 0
24 _____ 0 0 0
25 _____ 0 0 0
26 _____ 0 0 0
27 _____ 0 0 0
28 _____ 0 0 0
-Program Security-

APPENDIX F REACTOR PROCESS S AMPLING SYSTEM

COMPONENT DESCRIPTION

The permeable barrier reactor system was equipped with a pneumatic sampling system and 28 discrete sample points that were contained on two identical sample registers evenly distributed over the height of the unit. Each register contained 14 sample points. Twelve sample points on each register were positioned along the centerline of the reactor. Figure F.1 displays the spatial location of the sample points relative the unit inlet, outlet and treatment zones. From the inlet moving toward the outlet, sample point numbers jump from three to five. Sample points designated four-left and four-right are on the same plane as sample point two and were omitted from Figure F.1 for clarity. Sample points along the centerline allowed for the generation of longitudinal profiles while, those positioned in the mixing zone allow for characterization of one unique plane. Combination of sample points from the upper and lower manifolds allowed for complete spatial and temporal characterization of biological removal processes.

The pneumatic sampling system consisted of a gas distribution manifold, a sample loop with screened inlet, two check valves placed in opposition, a three-way valve and a regulated low pressure gas source. Each sample point operated on an independent channel and allowed the acquisition of discrete small volume samples. Individual sample points in the reactor were attached to a common control manifold. Separate manifolds were used to operate the upper and lower sample registers. Figure F.2 displays the overall piping schematic of the reactor sample system. Nitrogen gas was independently supplied to the upper and lower control manifolds through ¼" O.D. (6.4 mm) Teflon® tubing. The supply of nitrogen to each manifold was controlled using a three-way valve. The valves were configured with common outlets and two independent inlets. Manifold supply lines were connected to the outlet position of the valve. One inlet port was connected to the nitrogen supply while; the second inlet was left open to the atmosphere. On the control manifold, check valves at each sample channel were positioned to prevent the escape of nitrogen from the sample loop. The sample loop, connected to the control manifold consisted of a ¼" NPT female tee fitting. The branch of the tee was connected to another check valve that was also oriented to prevent gas escape. The run of the tee was connected to 1/8" O.D. (3.2 mm) Teflon® tubing which carried the sample into the process control trailer.

In normal operation, nitrogen gas was supplied to the control manifold at a pressure of 25psig. With the sample collection valves at the surface closed, a pressure of 25psig was attained in all sample channels. Check valve orientation on the branch of the tee prevented gas escape. However, it would allow liquid into the sample channel if the hydrostatic forces on the check valve spring exceeded the pressure in the sample loop. Using this principle, the supply of gas to the control manifold was terminated and the line pressure was equilibrated to atmospheric through rotation of the three-way valve. Check valve configuration still assured that 25 psig was present in each sample loop. Process samples were collected when the individual valves from each sample point were opened and equilibrated to atmospheric pressure. Following

equilibration on each sample channel, the pressure forcing the tee branch check valve closed became less than the exterior hydrostatic pressure which, allowed water to enter the sample loop. When the control manifold was returned to 25psig, liquid flow through the check valve into the sample loop ceased. Plug valves at the surface were throttled for each sample collected to allow for the displacement of gas and water contained in the tubing loop. The liquid sample expelled by the nitrogen gas was collected in a 4 ml amber vial and sealed with a Teflon® faced screw cap.

Construction plans for the sample control manifold are presented in Figure F.3. Locations of the sample registers in the permeable barrier reactor are detailed in Figure F.4.

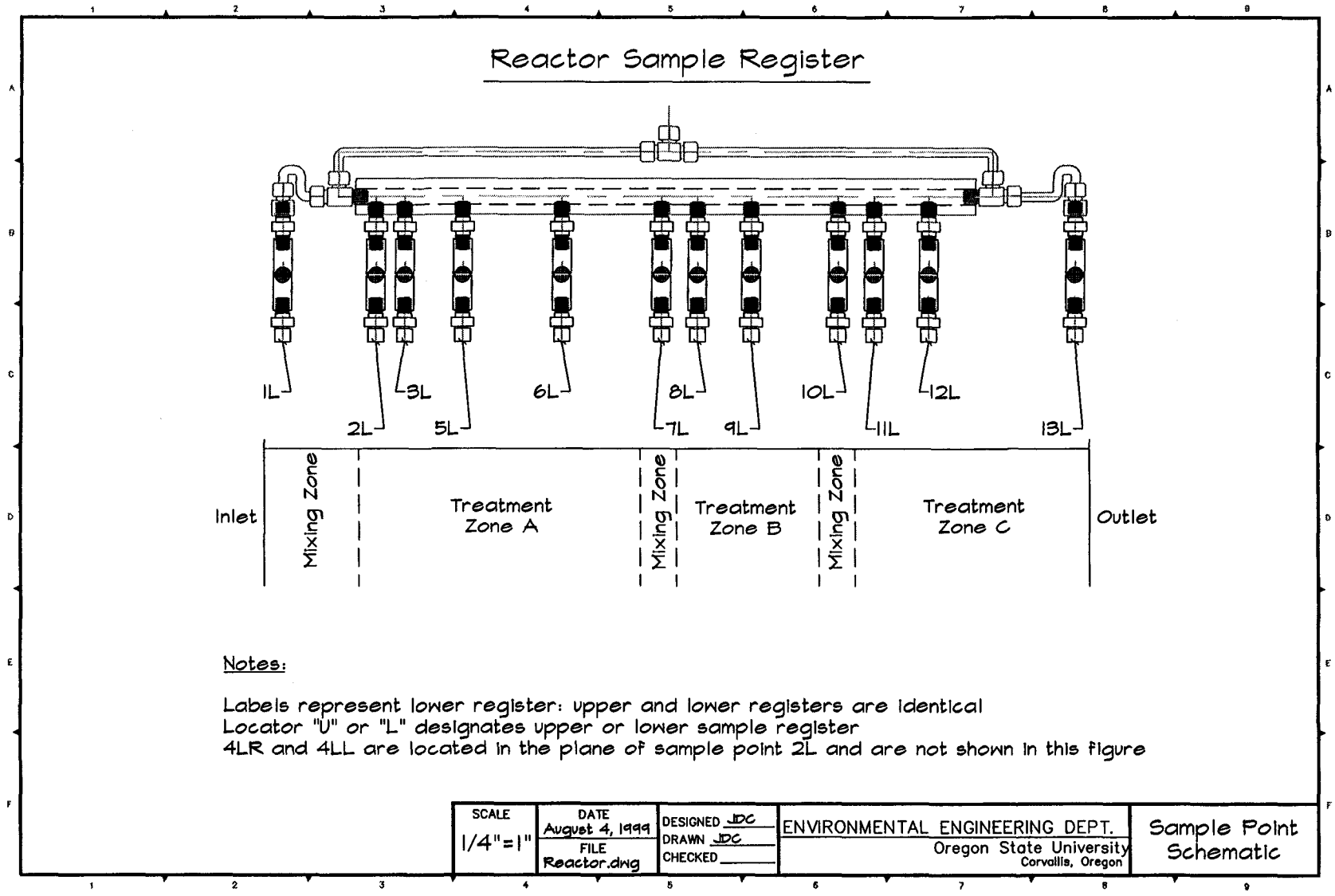

Figure F.1 Reactor sample register schematic

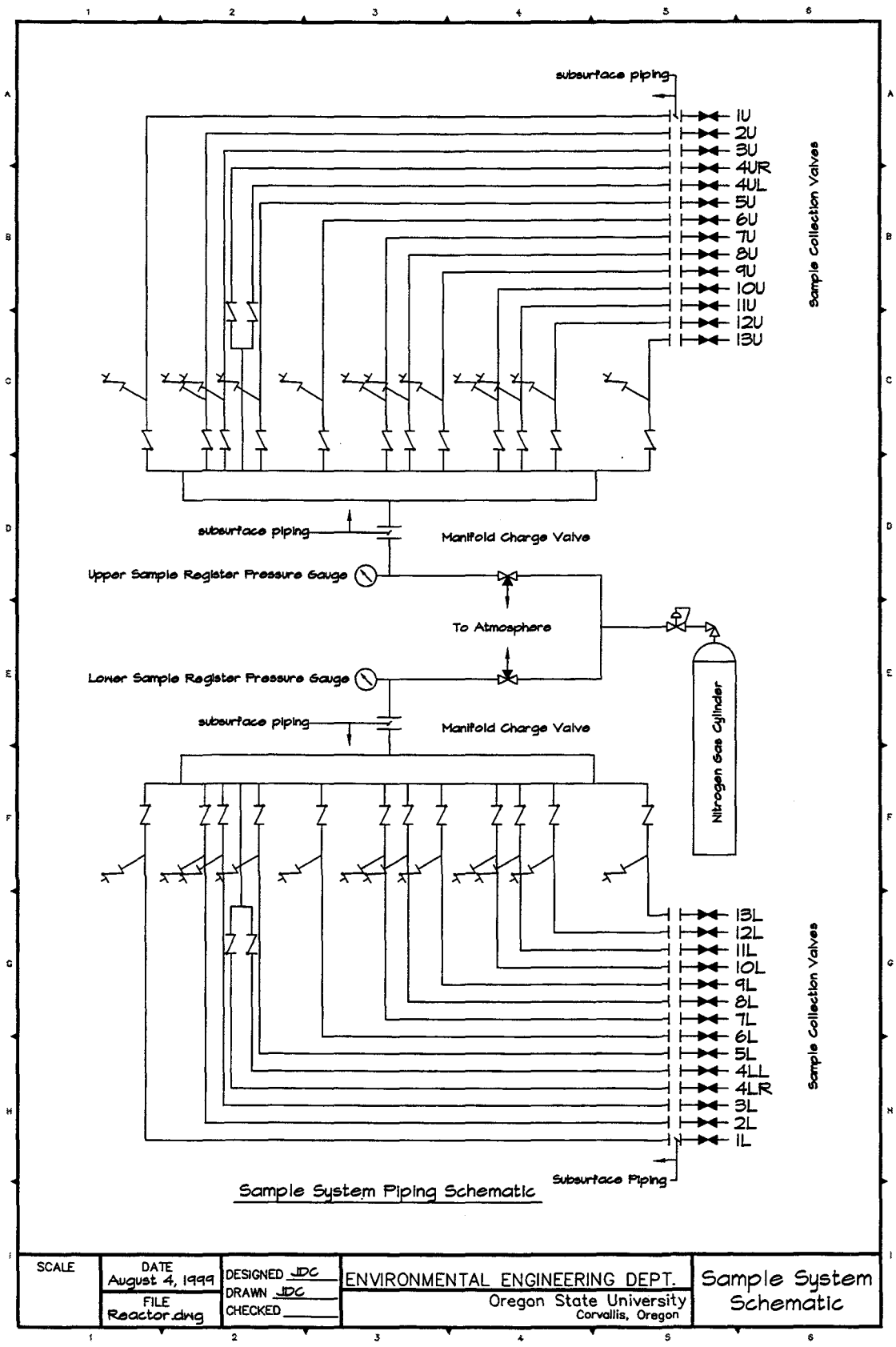

Figure F.2 Reactor sample system piping schematic

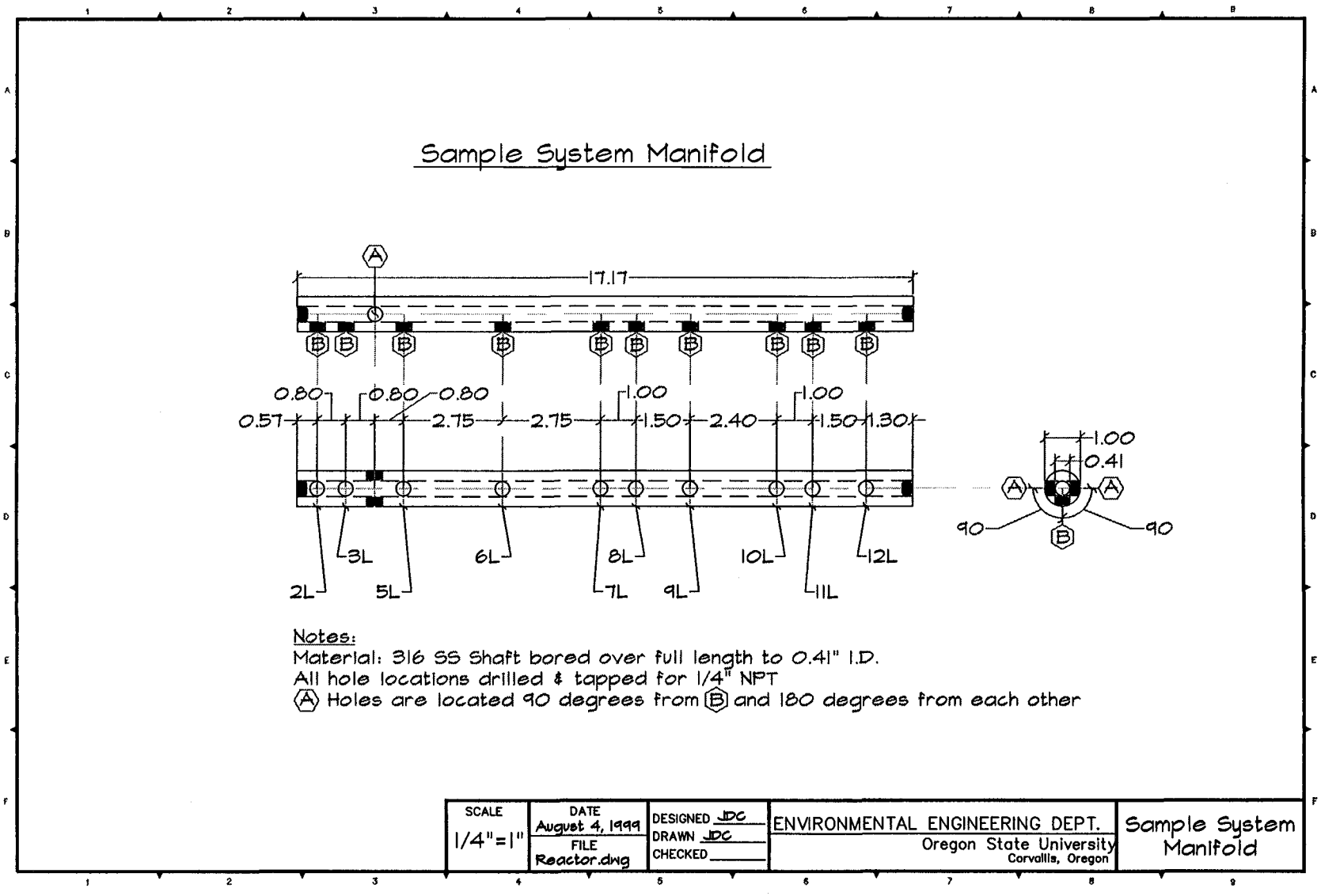

Figure F.3 Reactor sample manifold construction detail

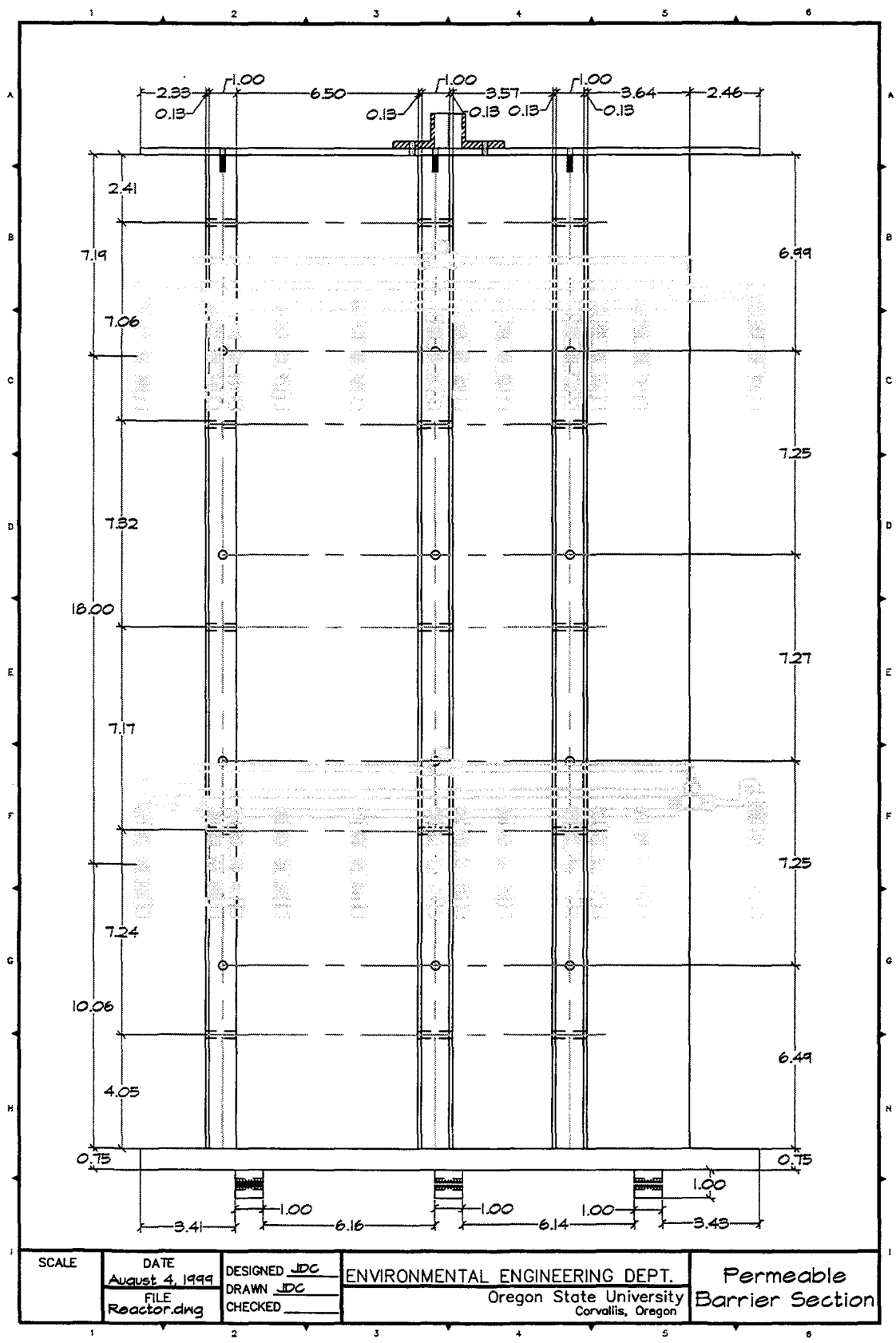

Figure F.4 Reactor sample manifold location plan

APPENDIX G FIELD SAMPLING PR O CEDURES: CHLOROPHENOLS

PURPOSE

To collect groundwater samples from an *in-situ* permeable barrier reactor operating at the McFarland Cascade site in Eugene, Oregon. There are 28 sample points over two levels embedded in the reactor. Numbers differentiate sample points, port one corresponding to the inlet and thirteen the exit. Letters modify sample port location, L referring to the lower sample register and U the upper. In addition, L and R are used to characterize left and right sample points installed on a unique plane in the first reactor mixing zone. Figure F.2 shows sample point location and naming system. Three small diameter-monitoring wells were also used to characterize aquifer conditions up and down gradient of the reactor assembly. Chlorophenols are quantified in the samples collected using the methods described in Appendix Q.

MATERIALS

Sample collection tower
Compressed inert gas cylinder & regulator
4 ml screw top amber glass vials
Teflon lined vial caps
Sample vial trays
Cooler and ice pack
Water (Reverse Osmosis Purified)
Nitrile gloves
Purge water storage vessel
Measuring tape with water level indicator

PROCEDURE

5. Label amber vials with a marking pen. Include the date, sample location and sample event e.g. (5L-29). Install labeled vials in plastic vial trays corresponding to the desired sample locations.
6. Check all sample port valves to ensure they are all fully closed. As you face the sample collection tower, port valve handles should be pointing to the left. Open the gas cylinder valve and charge the upper and lower registers by turning the gas supply valves fully to the right. Fully charged, the upper and lower register pressure gauges should read 20 psig or greater.
7. Install purge water collection trays on the sample tower under each sampling manifold. Purge each sample port by opening the valve counter clockwise to approximately the 8 o'clock position. Water will momentarily flow and eventually sputter out to a mixed gaseous and liquid stream. Turn the gas supply valves counter clockwise to release the upper and lower register charge gas. With the gas supply valve fully to the left, pause a few seconds, then close all sample port valves. Rotate the gas supply valves clockwise 180 degrees to recharge the upper and lower registers. Continue the purge

cycle by reopening the sample port valves. Again, water should momentarily flow from the sample ports.

8. Repeat the purge cycle three times. During the final purge, open each sample valve completely and allow the charge gas to flow and displace residual sample present in the line or valve body. Remove the purge water trays from the sample tower and empty to a storage vessel. When full, empty the storage vessel contents into the facility's storm water treatment system. Place the vial trays in their appropriate locations on the sample tower.

9. Turn each gas supply valve counter clockwise to release the upper and lower register charge. Fully open each sample valve, pause three to five seconds, then close. Repeat valve cycle for each sample location desired. When all sample valves have been cycled, rotate the gas supply valves clockwise 180 degrees to recharge the upper and lower registers. Throttle each sample valve to completely fill the 4 ml amber vials. Construct one field blank each sampling event by filling one 4 ml amber vial in the rack with RO water.

10. Remove the vial racks from the sampling tower, cap and place in a cooler on ice for transportation to the laboratory. Close the gas cylinder valve and turn the upper and lower register gas supply valves counter clockwise until the handles reach the 12 o'clock position. The upper and lower register gauges should read 20 psig or greater.

11. Using the 4 ml vials, obtain groundwater samples from the two up-gradient (MW98-1 and MW98-2) and the down-gradient (MW 98-3) monitoring wells. Samples are collected from a continuous flow loop system installed in the sample trailer. Rotate the valve handle counter-clockwise and allow water to purge momentarily. Without disrupting flow, place the appropriate vial in the stream and collect the sample, close the valve. Cap the vials and place on ice for transport to the laboratory for analysis.

12. Using the 4 ml vials, obtain groundwater samples from recovery wells R1 and R2. Samples are collected in the groundwater treatment building from ports installed on R1 and R2 discharge lines. Open the valves and allow water to purge momentarily. Without disrupting flow, place the vial in the stream and collect the sample, close the valve. Cap the vials and place on ice for transport to the laboratory for analysis.

13. Measure and record the water elevation in MW96-1 at the appropriate mark on the Northeast quadrant of the well casing.

APPENDIX H FIELD SAMPLING PR O CEDURES: SELECTED ANALYSES

PURPOSE

To collect and analyze groundwater samples from an *in-situ* permeable barrier reactor operating at the McFarland Cascade site in Eugene, Oregon. Process samples are collected from pinch valves installed downstream of the peristaltic pump used to supply the flow cell A and B. Three small diameter monitoring wells are also used to characterize aquifer conditions up and down gradient of the reactor assembly. Selected anions and cations in the samples are quantified using commercially prepared reagents and a portable colorimeter.

MATERIALS

Hach DR 890 Field Colorimeter
Hach Accuvac vials: nitrate
Hach Accuvac vials: sulfate
Hach Accuvac vials: ferrous iron
DR 890 Glass cuvette
Plastic 50 ml beakers
Water (Reverse Osmosis Purified)
Nitrile gloves
Purge water storage vessel
Hach Data link adapter
Hach Data link software
Lint free cloth

PROCEDURE

1. Using a clean 50 ml beaker, collect water samples from MW98-1, MW98-2, MW98-3 and each flow cell. Fill the glass cuvette with water taken from one of the sample locations.
2. Clear all stored data points from the memory of the DR 890 using the [setup] key. Begin with the nitrate analysis and load the factory calibrated method [prgm] [50]
3. Place a new nitrate Accuvac vial in each beaker with the neck pointed down. Apply firm pressure to the vial neck until it snaps. Verify that the neck is submerged while the vial fills. Once full, remove each vial from the beaker and invert the vials to mix. Using [timer] [enter] activate the one minute mixing timer. Once mixed, wipe the vials of all excess liquid and remove any fingerprints with a lint free cloth. Activate the five minute reaction period using the [timer] key.
4. Place the blank glass cuvette in the cell holder and cover; zero the colorimeter with a [zero] keystroke following the reaction period, the timer will sound. Place the nitrate sample in the cell holder; measure the concentration using [read]. Data is stored in the unit using the [store] keystroke. Analyze nitrate samples in the following order: MW98-1, MW98-2, MW98-3, Cell A and Cell B. Store the data points in registers one through five.

5. Continue the anion analysis with sulfate measurement. Load the factory-calibrated method with keystrokes [prgm] [92].

6. Repeat sample collection procedure and place a new sulfate Accuvac vial in each beaker with the neck pointed down. Apply firm pressure to the vial neck until it snaps. Verify that the neck is submerged while the vial fills. Once full, remove each vial from the beaker and invert several times to mix contents. Once mixed, wipe the vials of all excess liquid and remove any fingerprints with a lint free cloth. Activate the five minute reaction period using the [timer] [enter].

7. Place the blank glass cuvette in the cell holder and cover; zero the colorimeter with a [zero] keystroke. Following the reaction period, the timer will sound. Place the sulfate sample in the cell holder; measure the concentration using [read]. Store analysis information in the unit using the [store] key; enter [11] when prompted for the sample number. Analyze sulfate samples in the following order: MW98-1, MW98-2, MW98-3, Cell A and Cell B. Store the data points in registers eleven through fifteen.

8. Complete the analysis with measurement of ferrous iron. Load the factory-calibrated method with keystrokes [prgm] [33].

9. Repeat sample collection procedure and place a new ferrous iron Accuvac vial in each beaker with the neck pointed down. Apply firm pressure to the vial neck until it snaps. Verify that the neck is submerged while the vial fills. Once full, remove each vial from the beaker and invert several times to mix contents. Once mixed, wipe the vials of all excess liquid and remove any fingerprints with a lint free cloth. Activate the three minute reaction period using the [timer] [enter].

10. Place the blank glass cuvette in the cell holder and cover; zero the colorimeter with a [zero] keystroke. Following the reaction period, the timer will sound. Place the ferrous iron sample in the cell holder; measure the concentration using [read]. Store analysis information in the unit using the [store] key; enter [21] when prompted for the sample number. Analyze ferrous iron samples in the following order: MW98-1, MW98-2, MW98-3, Cell A and Cell B. Store the data points in register twenty-one through twenty five.

11. Collect used Accuvac vials and dispose in an approved container. Oregon State University's Environmental Health and Safety Extension handle ultimate disposal of these vials; waste pickup can be arranged when needed.

12. Transfer the field data from the DR 890 to a desktop computer using the infrared printing port adapter and the Hach-link software package.

APPENDIX I CARBONACEOUS OXYGEN DEMAND (COD) PROTOCOL

PURPOSE

To analyze groundwater samples for carbonaceous oxygen demand (COD) from an *in-situ* permeable barrier reactor operating at the McFarland Cascade site in Eugene, Oregon. Process samples are collected from pinch valves installed downstream of the peristaltic pump used to supply the flow cell A and B. Three small diameter monitoring wells are also used to characterize aquifer conditions up and down gradient of the reactor assembly. COD is measured using commercially prepared reagents and a portable colorimeter.

MATERIALS

Hach DR 890 Field Colorimeter
Hach TNT Adapter
Hach COD Digestion Tubes (0-150 PPM)
Hach COD Block Heater
Water (Reverse Osmosis Purified)
Nitrile gloves
Safety Glasses
2 ml fixed volume repeating pipette
Hach Data link adapter
Hach Data link software
Lint free cloth

PROCEDURE

1. Follow the guidelines established in Appendix G for collection of water samples from MW98-1, MW98-2, MW98-3, reactor points 7L, 7U and recovery wells R1 and R2.
2. Label each COD digestion tubes on the white marking panel and place in a test tube rack.
3. With safety glasses and gloves, remove the cap of the digestion vial. While holding the tube at a 45-degree angle, carefully pipette 2 ml of sample into the digestion vial and replace cap.
4. The digestion process is highly exothermic. Using insulated gloves, invert the digestion tube several times to mix the sample and reagents.
5. For each lot of digestion vials, create a blank using 2 ml of deionized water.
6. Place the digestion tubes in the Hach block heater and initiate the rotary timer to begin the two hour digestion reaction. Sample digestion is conducted at 150°C. Cooling time is required before the tubes can be handled for measurement.
7. Clear all stored data points from the memory of the DR 890 using the [setup] key. Install the TNT adapter into the light cell and load the factory calibrated method [prgm] [16] to measure COD concentrations.

8. Place the digestion blank in the cell holder and cover; zero the colorimeter with a [zero] keystroke Wipe the COD digestion vial to remove any fingerprints with a lint free cloth and place it in the cell holder; measure the concentration using [read]. Data is stored in the unit using the [store] keystroke. Analyze COD samples in the following order: MW98-1, MW98-2, MW98-3, 7L, 7U, R1 and R2. Store the data points in registers 31 through 37.

9. Collect used digestion vials and dispose in an approved container. Oregon State University's Environmental Health and Safety Extension handle ultimate disposal of these vials; waste pickup can be arranged when needed.

10. Transfer the field data from the DR 890 to a desktop computer using the infrared printing port adapter and the Hach-link software package.

APPENDIX J STANDARD TOTAL COLIFORM MEMBRANE FILTER PROCEDURE

PURPOSE

To enrich and quantify coliform bacteria present in groundwater samples collected from the McFarland Cascade site in Eugene, Oregon. Samples are evaluated for coliform by the approach detailed in Standard Methods. The following is a summary of method 9222 B (American Public Health Association, 1989).

MATERIALS

Sterile sample bottles
50 ml volumetric flask
100 ml Beaker
1 ml Volumetric pipette
Sterile disposable 10 ml pipettes
Sterile disposable petri dishes (50 x 12 mm)
Plastic filtration units
Suction flask
Kraft paper
Sterile membrane filters with grids
Filter forceps
Incubation chamber (35 ± 5 °C required)
Sterilized water (Reverse Osmosis Purified)
Dehydrated Difco M-Endo Agar LES (No. 0736)
Reagent grade 95% ethanol (not denatured)

PROCEDURE

1. Wrap plastic filtration units with heavy kraft paper sealing both ends. Sterilize the units, an aliquot of RO water and any vessel to be used for sample collection by autoclaving. Sterile filter units can be stored until use.

2. Collect samples for coliform analysis in any sterile container, 700 ml is sufficient. McFarland Cascade field samples are collected from a sample port installed on the head of recovery well R2. Open the valve and allow water to purge momentarily. Without disrupting flow, place bottle opening in the stream to collect the sample. Remove sample bottle before closing the sample port valve and take care not to touch the sample port with the sample collection bottle. The sample tap is labeled and located in the groundwater treatment building. Process the collected sample immediately.

3. Weigh 2.55 grams agar and mix with 1 ml ethanol in a 50 ml volumetric flask. Bring to volume with RO water and boil the solution to dissolve agar. Remove from heat and empty flask to a sterile 100 ml beaker. Using the disposable 10 ml pipette, dispense 7 ml to the bottom half of each petri dish. Seven petri dishes can be filled with 50 ml agar. Stack the dishes and allow the agar to gel before proceeding.

4. Assemble sterile filtration unit and place a sterile membrane filter with the grid side up on the filter base using sterile forceps. Carefully install funnel on the base taking care to not rip the membrane

filter. Filter 100 ml of sample through the assembly. Rinse the filter unit with 20-30 ml of sterile water. Relieve vacuum and remove filter with the forceps. Open a petri dish and lay the filter across the agar. Try to minimize air entrapment under the membrane. Cap the petri dish and look for uniform staining of the membrane after 2-5 minutes. Run five replicates of the sample collected from R2 and run two negative controls with 100 ml samples of sterile RO water.

5. Place the petri dishes inverted in an incubator set at 35 ± 5 °C for a period of 24 hours. Coliform present in the sample will grow in a circular fashion and possess a distinct metallic sheen with greenish tint. Record the number of colonies present in the collected samples and dispose of the petri dishes in a proper laboratory waste receptacle.

APPENDIX K PREPARATION OF IM I TATION VANILLA FLAVORING

PURPOSE

To prepare a stock feed solution for a mixed anaerobic culture of pentachlorophenol (PCP) degrading bacteria. Imitation vanilla flavoring will serve as an electron donor and PCP as the electron acceptor. This protocol describes steps required for the bulk manufacture of imitation vanilla flavoring. Table K.1 summarizes imitation vanilla flavoring constituents, concentrations and chemical unit costs.

Table K.1 Imitation vanilla flavoring components, concentrations and costs

Component	Formula (*g/L*)	Mass (*g*))	Cost (*$/g*)	Subtotal
Guaiacol	3.598	3.598	0.05	$0.18
Ethyl Vanillin	1.199	1.199	0.15	$0.18
Propylene Glycol	7.797	7.797	0.02	$0.16
Sodium Benzoate	0.840	0.840	0.01	$0.01
			Total Cost per liter	**$0.52**

MATERIALS

Top loading balance
20 Liter carboy
Water (Reverse Osmosis Purified)
Magnetic stir plate
Magnetic stir bar
500 ml wide mouth flask
Laboratory wax film

Reagents:
Propylene Glycol
Guaiacol (*o*-methoxy-phenol)
Ethyl Vanillin (3-ethoxy-4-hydroxybenzaldehyde)
Sodium Benzoate

PROCEDURE

1. On a top loading balance, dispense 155.9 grams of propylene glycol to a 500 ml wide mouth flask. With the flask on the balance, continue with the addition of 72 grams guaiacol, 24 grams ethyl vanillin and 16.8 grams sodium benzoate.

2. Add a magnetic stir bar and place flask on a stir plate. Cover flask with parafilm to prevent evaporation and stir until the solution is homogenous and lacks any suspended particles (3 to 5 hours is generally required).

3. Fill the 20 liter carboy with approximately 18 liters of RO water and place on a magnetic stir plate. Once the water is swirling, add the prepared concentrate in 100 ml doses to the water. To minimize concentrate precipitation, allow time between each dose for mixing. Rinse the concentrate flask with RO water several times with water, using the rinse water to bring the carboy to the 20 liter mark. Cap the carboy and allow the mixture to stir a minimum of 12 hours before use.

APPENDIX L IMITATION VANILLA FLAVORING BOTTLE STUDY PROTOCOL

PURPOSE

Develop a procedural method to determine the effectiveness and degradation pathway of pentachlorophenol (PCP) by an anaerobic culture when imitation vanilla flavoring serves as the electron donor.

MATERIAL

125 ml Amber serum bottles
Teflon faced butyl rubber stoppers
20 mm Aluminum crimp seals
20 mm Hand crimper
50 ml Graduated cylinder
Inert purge gas manifold
500 ml Erlenmeyer Flasks
Tubing sections
Small beakers
Sterile 18 ga. Needles
Assorted syringes with volumes of 50-500 μl
10 ml Ground glass syringe
Rotary shaking table at constant temperature
Reagents:
Anaerobic cell suspension
Sodium Bicarbonate (Na_2HCO_3)
S7 Vitamin solution
S4 Mineral solution
S3 Nutrient solution
Imitation vanilla flavoring (see Appendix K)
Pentachlorophenol

PROCEDURE

1. Acid wash the 125 ml amber serum bottles by soaking in 50% solution of sulfuric acid overnight. Rinse each bottle three times with RO and deionized water. Allow the bottles to dry and store in clean location for future use.

2. Assemble the media solutions required for the study. Appendix L summarizes the components of the media solutions and desired concentrations. The media solutions are duplicates of those used by Owen et al., (1979). Use of these solutions is widespread, so check for availability among fellow researchers before proceeding with the manufacture of solutions S4 and S7.

3. Using an appropriate volumetric flask, construct a stock solution of PCP in imitation vanilla flavoring. This solution allows the simultaneous addition of the electron donor/acceptor pair. Using the method outlined in Appendix Q, check the concentration of the solution before proceeding.

Table L.1 Stock solution contents and concentrations

S7 (Vitamins)		S4 (Minerals)	
Compound	**Conc. (mg/l)**	**Compound**	**Conc. (g/L)**
Pyridoxine Hydrochloride	10.00	$MgCl_2 \cdot 6H_2O$	120.00
Riboflavin	5.00	KCl	86.70
p-Aminobenzoic acid	5.00	NH_4Cl	26.60
Thiamin	5.00	$CaCl_2 \cdot 2H_2O$	16.70
Thioctic acid	5.00	$CoCl_2 \cdot 6H_2O$	2.00
Nicotinic acid	5.00	$MnCl_2 \cdot 4H_2O$	1.33
Pantothenic acid	5.00	$NiCl_2 \cdot 6H_2O$	1.00
Folic acid	2.00	H_3BO_3	0.38
Biotin	2.00	$CuCl_2 \cdot 2H_2O$	0.18
B_{12}	0.10	$Na_2MoO_4 \cdot 2H_2O$	0.17
		$ZnCl_2$	0.14
S3 (Nutrients)		$(NH_4)_2HPO_4$	26.70

1. Obtain a well mixed sample of the anaerobic culture and determine the concentration of volatile suspended solids using the method outlined in Appendix W. Based on a liquid volume of 90 ml in the serum bottles, compute the required volume of cells to provide approximately 800 to 1000 mg VSS.

2. Control bottles must be setup two days in advance of the active bottles to allow adequate sterilization time. Place a well mixed volume of the anaerobic culture in an 500 ml flask. Scribe the flask to mark the volume contained and cover with a foam plug. Obtain another flask and fill with de-ionized water. Autoclave the cells and DI water on three consecutive days for a period of 45 minutes. Use the sterile water to makeup volume lost from the cells during the autoclaving process.

3. Construct duplicate bottles and sterile controls for each experimental condition evaluated. Using a liquid volume of 90 ml, to each serum bottle, add 0.378 g Na2HCO3, 800 μl S7 vitamin solution, 1200

μl S4 mineral solution and 240 μl S3 nutrient solution. Label each serum bottle with the contents and experimental conditions. With a sterile needle, de-air the flask of autoclaved water with an inert gas.

4. Transfer a well mixed volume of cells to a large flask. Using a canula, continuously bubble an inert gas through the cell suspension. Measure the calculated volume of cells with a graduated cylinder. Quickly empty the contents into the serum bottle and place a second inert gas line into the serum bottle. Rinse the cylinder with the calculated volume of de-aired sterile water and add to the serum bottle. Cap the bottle with the butyl stopper and hand crimp the aluminum seal. Shake the bottle to thoroughly mix contents. Attach a small length of tubing to a small gauge needle. Place the tubing in a beaker of water and use the needle to equilibrate the serum bottle headspace pressure to atmospheric. Purge the headspace for several minutes with a needle attached to the inert gas supply. Remove the gas supply line and allow for atmospheric equilibration. Repeat the procedure for each bottle and control required.

5. When all bottles have been setup, sample the system for chlorophenols using the method outlined in Appendix Q. The data set will serve as the baseline condition for the experiment.

6. Spike each serum bottle with the imitation vanilla flavoring PCP mixture. Remove the vent line from the bottle and shake thoroughly. Record the time and immediately sample the system for chlorophenols using the method outlined in Appendix Q. The data set will serve as the initial experimental conditions. Place the serum bottles inverted on a rotary shaking table located in a constant temperature environment.

7. Sample the serum bottles on 12 hour intervals for chlorophenols to monitor system performance. Monitor and record gas production using a wetted 10 ml ground glass syringe. Gas composition can be determined by gas chromatography if desired.

APPENDIX M ELECTRON DONOR C O NCENTRATION BOTTLE STUDY PROTOCOL

PURPOSE

Develop a procedural method to determine the effect of electron donor concentration on the reductive dechlorination of pentachlorophenol (PCP) by an anaerobic culture harvested from the reactor demonstration well at the McFarland Cascade site in Eugene, Oregon.

MATERIALS

300 ml Wheaton Screw cap serum bottles
Screw cap closure with butyl rubber septum
250 ml Graduated cylinder
500 ml Erlenmeyer Flasks
15 ml Pipette and bulb
Assorted syringes with volumes of 50-1000 μl
Inert purge gas manifold
Sterile 22 ga. Needles
Constant temperature incubator (14°C)
10 ml Ground glass syringe
Reagents:
Groundwater from the McFarland Site
Imitation vanilla flavoring (see Appendix K)
Aqueous pentachlorophenol stock solution
Mercuric Chloride ($HgCl_2$)

PROCEDURE

1. Acid wash the 300 ml amber serum bottles by soaking in 50% solution of sulfuric acid overnight. Rinse each bottle three times with RO and deionized water. Cover the bottle openings with aluminum foil and sterilize the bottles and caps in the autoclave. Once cleaned, place the bottles in the airlock of the anaerobic glove box and cycle the system so they can be moved into the workspace.
2. Filter approximately 250 ml of a saturated aqueous PCP stock solution. Use a glass filter funnel and receiver unit with a Gelman type A/E glass fiber filter to perform the procedure. Using the method outlined in Appendix Q, check the concentration of the filtrate before proceeding.
3. Groundwater from the permeable barrier reactor at the McFarland Cascade facility in Eugene, Oregon serves as the inoculum and media for the bottle study. Groundwater is collected from the discharge of the two flow cells installed on site in the process control trailer. Blanket the headspace of a clean container with nitrogen gas and collect four liters of water. Continue the purging process until the container is full to promote anaerobic conditions.
4. Promptly remove the purge and fill lines from the container when full and quickly cap the vessel. Place the groundwater on ice for transport to the laboratory.

5. Upon arrival in the laboratory, loosen the cap on the groundwater vessel and immediately place the container in the air lock of the anaerobic glove box. Cycle the air lock and introduce the vessel into the workspace.

6. In the glove box, mix the groundwater vessel and dispense approximately 500 ml to an Erlenmeyer flask. Remove the flask through the airlock, cover with foil and autoclave. The aliquot of cells and groundwater removed will be used to set up parallel experimental controls.

7. When cooled, poison the control cell suspension with mercuric chloride and return the flask to the workspace of the anaerobic glove box.

8. In the glove box, homogenize the groundwater by thorough mixing. Label each serum bottle with the contents and experimental conditions. To each serum bottle, add 235 ml of the water using a graduated cylinder. For the control bottles, repeat the process using the sterilized groundwater. Construct duplicate bottles and sterile controls for each experimental condition evaluated.

9. Pipette 15 ml of the aqueous PCP stock solution into the serum bottles. Using a volumetric syringe, to each bottle add the appropriate volume of imitation vanilla flavoring to obtain the desired experimental conditions. Table M.1 summarizes the components and volumes added for each donor concentration evaluated.

Table M.1 Serum bottle contents and concentrations: electron donor concentration study

Treatment	Stock Conc.(mg/L)		Desired Conc. (mg/L)		Required Volumes		
	COD	PCP	COD	PCP	Media (ml)	PCP (ml)	Vanilla (μl)
0-	24250	20.2	0	1.29	235	15.01	0
10-	24250	20.2	10	1.29	235	15.01	103
25-	24250	20.2	25	1.29	235	15.01	258
50-	24250	20.2	50	1.29	235	15.01	515
100-	24250	20.2	100	1.29	235	15.01	1031
Control	24250	20.2	50	1.29	235	15.01	515

Note: Serum bottle assays were conducted in duplicate

8. Immediately following the addition of imitation vanilla flavoring, cap the serum bottles and shake several times to fully mix contents. Record the time and immediately sample the system for chlorophenols using the method outlined in Appendix Q.

9. Remove the serum bottles from the glove box and purge the headspace for several minutes with a needle attached to an inert gas supply. Remove the gas supply line from the septum and allow for atmospheric equilibration. Remove the vent line from the bottle and shake thoroughly. Repeat the procedure for every serum bottle constructed.

10. Place the serum bottles in an incubator at 14°C.

11. Obtain a well mixed sample of the groundwater and determine the concentrations of total and volatile suspended solids using the method outlined in Appendix W.

12. Sample the serum bottles on 12 hour intervals for chlorophenols to monitor system performance. Monitor and record gas production using a wetted 10 ml ground glass syringe. Gas composition can be determined by gas chromatography if desired.

APPENDIX N COMPETITIVE ELECT RON ACCEPTOR BOTTLE STUDY PROTOCOL

PURPOSE

Develop a procedural method to determine the effect of a competitive electron acceptor (sulfate $SO_4^{=}$) on the reductive dechlorination of pentachlorophenol (PCP) by an anaerobic culture harvested from the reactor demonstration well at the McFarland Cascade site in Eugene, Oregon.

MATERIALS

300 ml Wheaton Screw cap serum bottles
Screw cap closure with butyl rubber septum
250 ml Graduated cylinder
500 ml Erlenmeyer Flasks
15 ml Pipette and bulb
Assorted syringes with volumes of 50-1000 μl
Inert purge gas manifold
Sterile 22 ga. Needles
Disposable 1 ml syringes
Eppendorf micro-centrifuge tubes
Constant temperature incubator (14°C)
10 ml Ground glass syringe
Reagents:
Groundwater from the McFarland Site
Imitation vanilla flavoring (see Appendix K)
Aqueous pentachlorophenol stock solution
Sodium Sulfate (Na_2SO_4)

PROCEDURE

1. Move all serum bottles from the incubator to the work area inside the anaerobic glove box. Remove the serum bottle caps and replace septum. Set the bottles aside and allow for contents to fully settle.
2. Filter approximately 250 ml of a saturated aqueous PCP stock solution. Use a glass filter funnel and receiver unit with a Gelman type A/E glass fiber filter to perform the procedure. Using the method outlined in Appendix Q, check the concentration of the filtrate before proceeding.
3. Add imitation vanilla flavoring to the aqueous PCP stock solution in a volumetric flask to yield a COD of 10 mg/L. Obtain sodium sulfate from the chemical stock room and weigh the proper dosage for each electron acceptor condition evaluated.
4. Groundwater from the permeable barrier reactor at the McFarland Cascade facility in Eugene, Oregon serves as the inoculum and media for the bottle study. Groundwater is collected from the discharge of the two flow cells installed on site in the process control trailer. Blanket the headspace of a clean container with nitrogen gas and collect four liters of water. Continue the purging process until the container is full to promote anaerobic conditions.

5. Promptly remove the purge and fill lines from the container when full and quickly cap the vessel. Place the groundwater on ice for transport to the laboratory.

6. Upon arrival in the laboratory, loosen the cap on the groundwater vessel and immediately place the container in the air lock of the anaerobic glove box. Cycle the air lock and introduce the vessel into the workspace.

7. In the glove box exchange the water in the serum bottles with the freshly collected groundwater. Remove 200 ml of water from each of the serum bottles using a volumetric pipette taking care not to disturb the settled cell mass and replace with an identical volume of fresh groundwater. With the exception of the controls, repeat this procedure for each serum bottle evaluated.

8. Pipette 15 ml of the aqueous PCP and imitation vanilla stock solution into the serum bottles. Add the measured dosage of sodium sulfate to each bottle to obtain the desired experimental conditions. Table N.1 summarizes the components of the serum bottles and experimental conditions evaluated.

9. Amend the control bottles with the proper sulfate dosage.

Table N.1 Serum bottle contents and concentrations: competitive electron acceptor study

Treatment[1] e^- demand	Stock Conc.(mg/L)[2]		Desired Conc. (mg/L)		Bottle Composition		
	COD	PCP	COD	PCP	Media (ml)	PCP (ml)	Na_2SO_4 (mg)
0.5-x	170	20.2	10	1.29	240	15.01	2.82
1.0x-	170	20.2	10	1.29	240	15.01	5.64
2.0x-	170	20.2	10	1.29	240	15.01	11.27
5.0x-	170	20.2	10	1.29	240	15.01	28.18
10x-	170	20.2	10	1.29	240	15.01	95.90
Control	170	20.2	10	1.29	240	15.01	31.97

Notes: [1]Serum bottle assays were conducted in duplicate [2]Imitation vanilla flavoring and PCP mixture

13. Immediately following the addition of the sulfate salt, cap the serum bottles and shake several times to fully mix contents. Record the time and immediately sample the system for chlorophenols using the method outlined in Appendix Q.

14. Remove the serum bottles from the glove box and purge the headspace for several minutes with a needle attached to an inert gas supply. Remove the gas supply line from the septum and allow for atmospheric equilibration. Remove the vent line from the bottle and shake thoroughly. Repeat the procedure for every serum bottle constructed.

15. With a disposable 1 ml syringe and 22 ga needle, remove approximately 0.7 ml from the serum bottle. Discharge the liquid to an eppendorf micro-centrifuge tube. With the micro-centrifuge, spin the samples at 10,000 RPM for 6 minutes. Measure sulfate concentrations in each serum bottle using the Dionex 4000I ion chromatograph. Operation of the instrument is detailed in Appendix P.

16. Place the serum bottles in an incubator at 14°C.

17. Sample the serum bottles on 12 hour intervals for chlorophenols to monitor system performance. Monitor and record gas production using a wetted 10 ml ground glass syringe. Gas composition can be determined by gas chromatography if desired.

APPENDIX O THE EFFECTS OF HY D ROGEN PARTIAL PRESSURE ON 3,4,5-TCP DEGRADATION

PURPOSE

To evaluate the effect of hydrogen partial pressure on the reductive dechlorination of 3,4,5-trichlorophenol a computer controlled batch reactor (Stuart, 1996), was employed. The reactor was operated at various hydrogen headspace concentrations and 3,4,5-TCP degradation was measured with time. The reactor system was configured to measure and log pH and oxidation/reduction potential. Experiments were conducted at 13°C to mimic conditions present in the McFarland aquifer structure.

MATERIALS:

Serum bottles evaluated in Appendix M
Glass funnel
Disposable 1 ml syringes
Gas tight 500 μl syringe w/ valve
Micro-centrifuge tubes w/ glass liners
Vacuum pump
Glass filter funnel and receiver unit
Gelman type A/E glass fiber filters
Constant temperature incubator (13°C)
10 ml Glass syringe w/ 6" 22 ga. needles
Hach COD sample tubes
Batch reactor system
Blank 5-1/4" floppy disk
pH and E_H Standards
3,4,5-trichlorophenol stock solution
Compressed gas cylinders:
Hydrogen
1% hydrogen –99% nitrogen
Nitrogen
Carbon dioxide

PROCEDURE

1. Thoroughly clean the batch reactor vessel and examine all fittings on the reactor top for wear of signs of leakage. Pay special cleaning attention to ports used to spike chlorophenols; solvent washing may be necessary to ensure there is no chlorophenol carry over from the previous operating conditions. Replace all septum on reactor sampling ports. Assemble the reactor system and conduct a static leak test with water before proceeding.

2. Clean and inspect reactor electrodes. Soak platinum and pH electrodes in a 0.1 N solution of HCL for 30 minutes. Once cleaned, thoroughly rinse the electrodes and immerse the pH electrode in buffer solution of pH 7; soak the platinum half cell in a pH 7 buffer saturated with quinhydrone. Drain inner and outer junctions of the reference electrode rinse with deionized water and refill with the proper electrolytic solutions.

3. Attach the probes to their respective BNC connections on the computer interface box and pH meter. Calibrate the pH probe assembly with buffer solutions at pH 10 and 4. Disconnect probes and install in reactor top. O-ring sealing is facilitated through the use of silicon stopcock lubricant. Use the lubricant sparingly taking care not to contaminate probe-sensing areas.

4. Move the reactor system and all of the active serum bottles used in the sulfate study from the incubator to the work area inside the anaerobic glove box (see Appendix L for details of bottle construction).

5. In the glovebox, agitate the serum bottles and empty contents through a glass funnel into the batch reactor system. Repeat procedure and empty remaining serum bottles into the batch reactor system. Once full, seal the reactor filling hole and homogenize the contents by shaking.

6. Remove approximately 50 ml of reactor suspension and filter using a glass filter funnel and receiver unit with a Gelman type A/E glass fiber filter. Using the methods outlined in Appendix Q, Appendix P and Appendix I check the chlorophenol, sulfate and COD concentration of the filtrate.

7. Install reactor system in constant temperature incubator. Fasten electrode BNC convectors to computer interface box. Attach gas line from mass flow controllers to the appropriate inlet on reactor top. Attach reactor vent line to two water traps connected in series and VERIFY that the gas line is vented to the outdoors. Activate the magnetic stir plate to agitate reactor contents.

8. Install a blank floppy disk into the computer and start the program. Follow the information prompts and enter all required and pertinent data.

9. Set the mass flow controllers to provide the desired headspace gas concentration and flow rate. Enter the operational parameters from the mass flow controllers into the computer program. Verify headspace composition by gas chromatography using the method outlined in Appendix V.

10. Prepare two-polyethylene eppendorf centrifuge tubes and label date and time. In one tube place a glass insert. With the 10 ml glass ground syringe and 6" needle, open the reactor sample valve and withdrawal approximately two ml of liquid. Fill the glass sleeve with liquid and dispense the remaining liquid to the other centrifuge tube. With the micro-centrifuge, spin the samples at 10,000 RPM for 6 minutes.

11. From the glass lined centrifuge tube sample chlorophenols in duplicate using the procedure outlined in Appendix Q. Immediately load the samples for chromatographic analysis using the guidelines in Appendix R or Appendix S. With the remaining centrifuge tube, measure the concentrations of major anions in the reactor system using the Dionex 4000I ion chromatograph. Operation of the instrument is detailed in Appendix P.

12. Sample the reactor system for chlorophenols, anions and headspace composition as previously described on two hour intervals (more frequently if required) to evaluate the kinetics of chlorophenol

removal. After chlorophenol degradation has been established, alter the hydrogen concentration present in the reactor headspace. Evaluate changes in the kinetics of chlorophenol degradation.

APPENDIX P OPERATION OF THE DIONEX 4000I ION CHROMATOGRAPH

PURPOSE

Develop a procedure for the measurement and interpretation of anions by ion chromatography. This protocol was adopted from operation guidelines established by laboratory manager Mohammed Azizian.

MATERIALS

Dionex 4000I Icon Chromatograph
HPIC-AS4A Column
One ml plastic syringe for manual injection
Dionex polyvials & caps for auto- injection

Regenerant Solution: 2L DI H2O
1.45 ml conc. H2SO4
Eluant Solution: 2L DI H2O
0.382 g Na2CO3 (anhyd.)
0.286 g NaHCO3

PROCEDURE

1. Fill in the IC logbook with information including your name, date, number of samples and any additional information about parameter settings, performance, needed maintenance.
2. Each day make a fresh eluant and regenerant solutions before running instrument using the labeled 2L volumetric flasks on the bench near the IC. Old solutions can support microbial growth, which will decrease column life.
3. The regenerant solution is contained in a 5L container on the countertop and labeled "Anion Regenerant". Eluant solutions are contained in the six 2.5 L reservoir bottles located in the rack on top of the IC instrument:
4. Reservoir bottle No. 1 = deionized water (DI H_2O)
5. Reservoir bottle No. 2 = anion eluant
6. Fill reservoir bottles No. 1 and No. 2 and tighten cap.
7. Tighten screw cap for anion regenerant bottle.
8. Turn He and N2 main tank valves on and check for adequate supply pressure. The regulators are preset to 40-60 psig respectively; DO NOT change the line pressures set on the regulator.
9. For anions, rotate the anion regenerant regulator valve clockwise to 5 psig.
10. Set the DEGAS module: Leave DEGAS module settings for at least 15 minutes before next step.

    Degas switch to HIGH;
    System switch to ON;
    Sample switch to ON;
    #1 switch to ON;
    #2 switch to ON.

11. After at least 15 minutes, set Degas switch to LOW; Check regenerant valve used to make sure that it reads 5 psig.
12. Select program to run by pressing [pgm] followed by the appropriate number on the front panel of the Gradient Pump Module:

    4 = Anion analysis
    3 = Anion column clean-up

13. Set conditions on Conductivity Module: For anions, a stabilized reading should be 14-16 μS

14. Set Output Range for detector at 30;
15. Set Temperature compensate to 1.7 (preset—don't change);
16. Turn Conductivity Cell to ON and wait about 5 minutes for a stabilized reading.

MANUAL OPERATION

1. Filter samples (excluding standards made with DI H2O) through a 0.2 u membrane filter to remove organic material. A 13 mm Swinney filter (Gelman #4317) and 13 mm Gelman Nylaflo disposable filters are recommended to filter 0.5 ml samples.
2. On the integrator panel, press [use file] followed by 5 and [enter]
3. Disconnect the autosampler line from injection port and install luer-lock adapter.
4. Inject the sample into injection port (0.5 ml or greater).
5. Press [inj a], sample will now be running. Repeat steps 4 and 5 for remaining samples.

AUTOMATED OPERATION

1. Pipette about 600 μl of sample or standard into Dionex polyvials. This should bring sample above the vial's 0.5 ml line. Press the black polyvial cap (white end down) into vial using the black plastic tool. There should be little or no entrapped air in the vial. If there are any bubbles clinging to the side of the vial, tap vial gently on lab bench or against your palm.
2. Prepare a DI H2O blank in the same manner.
3. Label each vial with a marking pen.
4. Load the DI H2O blank in the first position of the sample rack (hole closest to the white dot) followed by samples and standards in order of increasing concentration.
5. Open lid and load the left half of autosampler by pushing spring-loaded bar away from you and inserting sample racks. Line up the white dots of the racks facing the right side. Do not load more than 8-9 racks at a time.
6. Close lid and set autosampler to run by pressing [run/hold] button.
7. On the integrator panel, press [use file] followed by [0] and [enter]
8. Select [1] for anion analysis followed by [enter].
9. Press [inj b] samples will now be run automatically.

SHUT DOWN PROCEDURE

1. Deactivate pump by pressing [stop].
2. Select program to clean IC column by pressing [pgm] followed by the appropriate number on the front panel of the Gradient Pump Module.
3. 3 = Anion column clean-up program (this pumps DI H_2O only through the column)
4. Start pump by pressing [start] and let pump run for 30 minutes.
5. Stop pump by pressing [stop].
6. Turn conductivity cell off by pressing [off].
7. Turn main tank valves for He and N2 off.
8. Turn regenerant regulator valve off (counter-clockwise).
9. Turn off all gas module switches.
10. Release pressure in regenerant container and eluant reservoirs by unscrewing caps.
11. Used polyvials are not reusable and should be disposed.

APPENDIX Q CHLOROPHENOL ASS A Y PROTOCOL

PURPOSE

Develop a procedural method to analyze chlorophenols with a gas chromatograph (GC). The method originally was developed by Voss *et al.* (1981) then modified by Perkins (1992) and later miniaturized by Dr. Mark Smith (Smith, 1993).

MATERIALS

100 µl adjustable volume repeating pipette
500 µl adjustable volume repeating pipette
1000 µl fixed volume repeating pipette
Pasteur pipettes & bulb
Disposable 10 ml screw top culture tubes
Teflon® lined culture tube caps
2 ml Amber Autosampler vials
11 mm Aluminum crimp Viton® lined caps
Hand crimper
Wrist action shaker
Chlorinated waste container
Rotary Evaporator
Fume Hood
Acetic anhydride, reagent grade
Hexane, HPLC grade
Methanol, reagent grade
Chlorophenol reagent:
30.4 g/l potassium bicarbonate (K_2CO_3)
500 µg/l 2,4,6-tribromophenol (TBP)

PROCEDURE

1. To each test tube add: 500 µl of the chlorophenol reagent with a repeating pipette, an appropriate sample volume using a clean syringe (typically 100 µl) and 100 µl of acetic anhydride with the appropriate repeating pipette. When dispensing, be careful not to contaminate the syringe or pipette tips by dispensing along the culture tube walls.
2. Cap each culture tube and invert several times by hand to promote mixing then place on a wrist action shaker for 20 minutes.
3. Remove the tubes and uncap, a small release of gas is a normal occurrence so use caution. If no gas is emitted from the tube, it is probable that the acetic anhydride was not added to the culture tube.
4. To each culture tube add hexane using the 1 ml fixed volume repeating pipette. Recap the tubes and invert several times by hand then place them on the wrist action shaker for an additional 20 minutes.
5. With a marking pen, label an autosampler vial for each extraction conducted. Do not use tape to label vials it may hinder autosampler operation.

6. Remove the culture tubes and examine each for the presence of two phases. If this does not exist, the hexane was not added to the tube. Using a new Pasteur pipette for each sample, remove the top fraction of hexane from each tube and fill the appropriate autosampler vial. Finally, seal the autosampler vials with Viton® faced aluminum crimp caps.

7. Samples may be stored in the refrigerator or immediately loaded onto autosampler carrel for gas chromatographic analysis as described in Appendix R or Appendix S.

8. Obtain an empty four liter amber glass solvent bottle. Label the bottle with you name, the date and contents e.g. "chlorinated phenols in hexane and water". To each 4 liter container needed, add 500 grams of technical grade potassium chloride. After each sample has been prepared, the liquid remaining in each culture tube should be emptied into the appropriate waste container.

9. When the waste bottle is full, use a large separatory funnel to separate the hexane and water fraction. Collect the water on the bottom of the funnel and waste to a sink drain. Drain disposal of the separated water is possible because chlorophenols are very hydrophobic; subsequently the concentration of chlorophenols in the water is negligible. Dispense the hexane remaining in the funnel to another empty 4 liter bottle, label e.g. "chlorinated phenols in hexane" and store in a solvent cabinet.

10. When volumes of the collected contaminated hexane exceed 2 liters, solvent reclamation is required. Purified hexane is reclaimed from the waste container by distillation in rotary evaporation system. Install the rotary evaporator in the fume hood. Fill the water bath, set the temperature to 80°C and activate heater coil. Connect a vacuum line from a sink aspirator to the condenser body and a cold water supply to the condenser cooling coils.

11. Once the water bath is heated, fill the lower boiling flask with the contaminated hexane. Lower the condenser and rotating arbor into the water bath to immerse the boiling flask. Activate the aspirator to produce a vacuum in the condenser body and coolant supply to condenser coils. Set the evaporator to rotate at 50 revolutions per minute and watch for accumulation of clean condensate in the condenser receiving flask.

12. When the receiving flask is full, stop rotational movement of the boiling flask and remove the assembly from the water bath. Open the ground glass petcock to relieve the vacuum in the condenser assembly and remove the receiving flask. Empty the flask contents to an appropriately labeled container and store for use or disposal. Remove the concentrated foul hexane from the boiling flask and empty contents to an appropriately labeled container for disposal.

13. Oregon State University's Environmental Health and Safety Extension handle ultimate disposal of all hexane wastes; waste pickup can be arranged when needed.

APPENDIX R OPERATION OF HP 58 90 GAS CHROMATOGRAPH

PURPOSE

Analysis of chlorinated phenols by capillary gas chromatography.

MATERIALS

HP 5890 GC w/ Electron Capture Detector
HP Automatic Liquid Sampler
HP 3392 Integrator
Splitless inlet configuration
Capillary Column (30m x 0.32 mm x 0.25μm 5% Phenylmethyl Silicone Film)

PROCEDURE

1. Load the autosampler vials and record the sample information and vial location. To insure clean instrument operation, run one clean hexane sample for every 6 samples analyzed.
2. Fill out the logbook for the GC. Enter your name the date, the analysis method, the number of samples, and the resting detector signal. Check gas supply manifold and record tank pressures for all instrument gases. Do not attempt to adjust the supply line pressure of any gas located in the manifold assembly. Replace any cylinder that contains less than 300 lbs of gas. Record any instrument maintenance or configuration changes.
3. Check ECD makeup gas valve to insure it is operating; the argon/methane mixture should be supplied at flow rates no less than 75 ml/min when using a capillary column. Check the column head pressure, 14 psig (linear He velocity of 35 cm/s) is recommended for chlorophenol analysis.
4. Note the location and function of the vials on the automatic liquid sampler turret and drain each to an appropriate disposal container. Replace the empty waste vials and refill solvent vials A and B with methanol and hexane respectively.
5. Workfile 1 is used for chlorophenol analysis and is automatically loaded when the keystrokes [WORKFILE] [1] [ENTER] are initiated on the HP 3392 integrator. Confirm the following temperature setpoints with keystrokes on the HP 5890 GC:

   Detector temperature 350°C & Injector Temperature 250°C
   Purge Activation ON 0.50 minutes (splitless injection)
   Initial temperature 40°C hold for 1 minute

Ramp 25°C/minute to 140°C

Ramp A 10°C /minute to 250°C hold for 5 minutes

6. To activate the autosampler, press [OPTION] [11] on the HP 3392 integrator. Answer the following questions pertaining to the automatic liquid sampler by pressing enter to make no changes; the value to make changes. At this time, the GC program method will be displayed. List the method file on the integrator by pressing [LIST] [WORKFILE] [1] then, start the GC run with the [START] key on the instrument keypad.
7. Remove chromatograms from the integrator.
8. Remove sample vials from the autosampler tray. Store sample vials in the freezer for future analysis or dispose in an approved container. Oregon State University's Environmental Health and Safety Extension handle ultimate disposal of these vials; waste pickup can be arranged when needed.

APPENDIX S OPERATION OF HP 6890 GAS CHROMATOGRAPH

PURPOSE

Analysis of chlorinated phenols by capillary gas chromatography.

MATERIALS

HP 6890 GC w/ Electron Capture Detector
HP Automatic Liquid Sampler
HP Chemstation
Splitless inlet configuration
Capillary Column (30m x 0.32 mm x 0.25μm 5% Phenylmethyl Silicone Film)

PROCEDURE

1. Open communication between the Chemstation and the instrument and load the appropriate sequence table.
2. Load the autosampler vials and record the sample information and vial location in the sequence table. Define sequence to run one clean hexane or one blank injection for every 6 samples analyzed.
3. Fill out the logbook for the GC. Enter your name the date, the analysis method, the number of samples, and the resting detector signal. Check gas supply manifold and record tank pressures for all instrument gases. Do not attempt to adjust the supply line pressure of any gas located in the manifold assembly. Replace any cylinder that contains less than 300 lbs of gas. Record any instrument maintenance or configuration changes.
4. Check ECD makeup and anode purge gas flows to insure they are operating; the argon/methane mixture should be supplied at flow rates no less than 60 ml/min and 6 ml/min respectively. Check the linear velocity of helium on the column, 35 cm/s is recommended for chlorophenol analysis.
5. Note the location and function of the vials on the automatic liquid sampler turret and drain each to an appropriate disposal container. Replace the empty waste vials and refill solvent vials A and B with methanol and hexane respectively. Confirm the following temperature setpoints with keystrokes on the HP 6890 GC or view method within the Chemstation under instrument methods:

   Detector temperature 350°C & Injector Temperature 250°C
   Purge Activation ON 0.50 minutes (splitless injection)
   Column carrier gas constant flow at 35 cm/s
   Initial temperature 40°C hold for 1 minute

Ramp 25°C/minute to 140°C

Ramp A 10°C /minute to 250°C hold for 5 minutes

6. Check for adequate printer paper. To activate the autosampler and begin the sample run, start the sequence with the Chemstation software.

7. Once all samples have been analyzed, remove the generated data files from Chemstation hard drive using the attached ZIP drive.

8. Remove sample vials from the autosampler tray. Store sample vials in the freezer for future analysis or dispose in an approved container. Oregon State University's Environmental Health and Safety Extension handle ultimate disposal of these vials; waste pickup can be arranged when needed.

APPENDIX T STANDARD CURVE PROTOCOL

PURPOSE

Develop a standard calibration curve specific to chlorophenols for gas chromatographic analysis. The calibration curve is designed to mathematically relate compound's peak area to it's molar mass. The curves are generated from chlorophenol standards of known concentrations. Third party chlorophenol standards were prepared under special order by Ultra Scientific, Inc. (North Kingston, RI). To minimize co-elution of peaks in the standards, the chlorophenol suite was segregated into two standards (No. 1 and No. 2). The standards are individually packaged in flame sealed ampoules for the expressed purpose of standard curve generation. The standard in methanol is highly concentrated, thus to accurately measure small sample volumes, a dilution is required. Contents and associated concentrations of each standard are listed Table T.1.

MATERIALS

See chlorophenol assay protocol

PROCEDURE

1. Crack the glass ampoule and transfer contents to a 4 ml amber vial with a new pasteur pipette. Using the pure standard, create a one hundred-fold dilution with GC grade methanol. Standards No. 1 and No. 2 are packaged in 2 ml and 1 ml aliquots. Therefore, proper dilution of No. 1 and No. 2 will require volumetric flasks of 100 ml and 50 ml, respectively. Transfer the diluted standards to several 4 ml amber vials. Seal the pure and diluted standard vials with Teflon lined caps.
2. Using the chlorophenol assay outlined in Appendix Q extract sample volumes of 25, 50, 100, 250 and 500 μl from the diluted standards and 10 μl from the pure standards. Create at least one method blank to ensure clean reagents and laboratory practices.
3. Using the sample volumes and the given standard concentrations in Table T.1, the respective masses in moles of each GC injection can be computed. Since the assay is based on a 100 μl extraction (Appendix Q), normalize the mass of standard extracted to 100 μl.
4. HP 5890 Appendix R: Analysis of the GC data is facilitated through the use of a computer spreadsheet package. The area ratio, (the area of the chlorophenol divided by the area of the internal standard), is plotted on the ordinate and the molar mass on the abscissa. The ultimate goal is to create a graph relating the mass in moles of chlorophenol injected to the area ratio. From the graph, the unknown concentrations of chlorophenol samples can be determined.

5. HP 6890 Appendix S: Use the resulting chromatograms to construct a method calibration table. Follow the instructions on the Chemstation to create a new calibration table. Enter the names and appropriate concentrations for the analytes and allow the computer to generate response factors for the compounds present in the standard. The software will plot calibration curves for each entered analyte. Choose a curvilinear calibration curve forced through zero when analyzing chlorophenols with an ECD cell. Save the table and update the instrument method. Future runs should automatically display sample concentration on the chromatogram.

6. Random standard points should be run with unknown samples routinely to ensure accurate instrument calibration and representative standard curves. Repeat the calibration procedure if excessive concentration deviation is observed or at a minimum once per month.

Table T.1 Chlorophenol standards

Compound		**Standard Solution Concentration (mg/L)**			
Std. No. 1	**Std. No. 2**	**No. 1**	**1:100 No. 1**	**No. 2**	**1:100 No. 2**
3-CP	2-CP	320.4	3.204	320.4	3.204
3,4-DCP	4-CP	201.0	2.010	320.6	3.206
3,5-DCP	2,6-DCP	200.8	2.008	200.6	2.006
3,4,5-TCP	2,4-DCP	100.2	1.002	200.8	2.008
2,3,5-TCP	2,3-DCP	100.0	1.000	200.8	2.008
2,3,4,5-TCP	2,4,6-TCP	60.0	0.0600	100.4	1.004
2,3,5,6-TeCP	2,3,6-TCP	60.2	0.0602	100.2	1.002
PCP	2,4,5-TCP	40.0	0.0400	100.2	1.002
	2,3,4-TCP			100.2	1.002
	2,3,4,6-TeCP			60.2	0.0602
	PCP			40.2	0.0402

APPENDIX U RETENTION TIMES F O R CHLOROPHENOLS

Table U.1 Chlorophenol retention times - expected & relative

Compound	Expected Retention Time (min.)	Relative Retention Time (min.)
2-CP	6.298	0.570
3-CP	6.505	0.588
4-CP	6.563	0.594
2,6-DCP	7.292	0.660
2,4-DCP	7.476	0.676
3,5-DCP	7.585	0.686
2,3-DCP	7.740	0.700
3,4-DCP	7.986	0.722
2,4,6-TCP	8.296	0.750
2,3,6-TCP	8.718	0.789
2,3,5-TCP	8.789	0.795
2,4,5-TCP	8.846	0.800
2,3,4-TCP	9.272	0.839
3,4,5-TCP	9.410	0.851
2,3,5,6-TeCP	9.994	0.904
2,3,4,6-TeCP	10.041	0.908
2,3,4,5-TeCP	10.626	0.961
2,4,6-Tribromophenol	11.055	1.000
Pentachlorophenol	11.779	1.065

APPENDIX V OPERATION OF HP 58 9 0 SERIES II GAS CHROMATOGRAPH

PURPOSE

Analysis of headspace gas composition using gas chromatography.

MATERIALS

HP 5890 Series II GC w/ thermal conductivity detector (TCD)
HP Chemstation
Packed column inlet configuration
Column for gas separation (H_2, N_2, O_2, CH_4)

PROCEDURE

1. Load the method on the HP 5890 control pad the following keystrokes [Load][1]. Verify that TCD reference gas supply is on and that sensitivity is set to [High]. Allow 30 minutes for signal stabilization if the instrument has been off. A stable signal in the range of 1.0 to 2.0 indicates the instrument is ready for sample analysis.
2. Open communication between the Chemstation and the instrument. Load the method for data analysis and complete the information required in the sample information table.
3. Collect the headspace sample using a 500 μl gas tight syringe and analyze immediately. Ready lights will indicate that the instrument is ready to accept the sample. Pierce the septum of the injection port and quickly expel syringe contents. Concurrent to sample injection press [Run] on the HP 5890 keypad.
4. Fill out the logbook for the GC. Enter your name the date, the analysis method, the number of samples, and the resting detector signal. Check gas supply manifold and record tank pressures for all instrument gases. Do not attempt to adjust the supply line pressure of any gas located in the manifold assembly. Replace any cylinder that contains less than 300 lbs of gas. Record any instrument maintenance or configuration changes.

APPENDIX W TSS & VSS ANALYSIS PROTOCOL

PURPOSE

To quantify the amount and type of solids present in an effluent sample taken from an anaerobic continuous stirred tank reactor. Samples are passed through a pre-weighed glass-fiber filter, dried, and reweighed. The weight after drying at 105°C, divided by the sample volume represents the total suspended solids. Once weighed, samples are introduced into a high temperature furnace to drive off all volatile organic compounds. The difference in weight following removal from the furnace divided by the sample volume represents the volatile suspended solids concentration. Samples are analyzed by the approach detailed in Standard Methods. The following is a summary of method 2540 D and 2540 E (American Public Health Association, 1989).

MATERIALS

Inert weighing dishes (one for each sample)

Muffle Furnace for operation at 500 ± 50°C

Drying oven for operation at 103 to 105°C

Desiccator with moisture indicator

Analytical balance

Glass-fiber filters (Gelman Science type A/E)

Filtration apparatus

Graduated cylinder

Distilled/deionized water wash bottle

Suction flask

Forceps

PROCEDURE

1. Before a sample can be analyzed, a filter must be prepared. Assemble the filtration apparatus and using forceps, carefully install a glass-fiber filter wrinkle side up, on to the filter plate. Apply a vacuum and wash the filter at least three times with 20 ml of distilled/deionized water.
2. Once rinsed, remove the filter with forceps and place it in an inert weighing dish. Place the wet filter on the side of the dish so that it does not adhere to the dish while drying. Each dish should be pre-marked (preferably etched) so that future identification is facilitated. The use of ink or grease pencils is not recommended.
3. Place the filters in a drying oven overnight, cool in a desiccator, and weigh. Record the weight and transfer the filters to a desiccator with adequate moisture adsorption capability for storage.
4. Begin sample analysis by assembling the filter apparatus and applying a vacuum. Remove the prepared filters and dishes from the desiccator and place on the filter plate using forceps.

5. Wet the filter and assembly with distilled/deionized water to allow for proper filter seating. Using a graduated cylinder, introduce a well mixed representative sample into the filtration apparatus and record the volume. Use a constant sample volume if duplicate runs are used.

6. After initial sample filtration, rinse the graduated cylinder completely to remove any solids that may have adhered to the glass walls. Wash the filter apparatus at least three times with distilled/deionized water. Allow for complete removal of water before each successive rinse.

7. Continue suction for at least three minutes to ensure complete filtration. Remove the filter and place in the appropriate dish so that filter adhesion is avoided. Place in a 103oC to 105oC drying oven overnight then transfer to a desiccator for cooling. When sample temperature approaches that of the balance, weigh all the samples and record the appropriate data.

8. The difference in weight divided by the sample volume introduced is the value of the total suspended solids.

9. If volatile suspended solids are also desired, immediately introduce the residual sample and dishes into a muffle furnace for 20 minutes.

10. Initially allow atmospheric cooling. However, as balance temperature approaches, transfer all samples to a desiccator for final cooling. Weigh the cool samples and record the difference in weight. The difference divided by the sample volume represents the volatile solids fraction of the sample. In both test cases, if duplicate samples are used, samples should agree within 5 % of their average. For TSS and VSS analyses, a minimum of triplicate samples should be processed.

APPENDIX X FIELD TRACER STUD IES

INTRODUCTION

Successful application of a permeable barrier treatment strategy requires a through understanding of aquifer system hydraulics. The barrier, which is installed within the aquifer, is in essence a process reactor. Regardless of unit operation employed in the reactor several constraints must be estimated and evaluated. Of these physical constraints, none possess more importance than the systems residence time. Whether the reactions occurring are biological, chemical, or physical, the mean residence time available for reactions must be determined.

Over the course of the field demonstration project, several attempts were made to characterize the retention time through the use of a conservative groundwater tracer. Based upon the unique nature of the pilot scale reactor and site conditions, an iterative approach for tracer release and data analysis was required. In the absence of a suitable upgradient injection point, a sodium bromide solution was introduced into the reactor assembly. Bromide concentrations were monitored with time and space, assuming the treatment unit operated under a plug hydraulic regime. Data analysis of early tracer releases failed to support plug flow operation therefore; tracer delivery methods were modified to allow for data analysis typical of completely mixed flow conditions. Five methods of tracer solution delivery were evaluated before a method was chosen for the comprehensive tracer test. As such, only results from tracer test six and seven are presented.

MATERIALS & METHODS

Tracer Test No. 6: Fall 1997

Sensing the limitations of the pneumatic sample system used in tracer tests one through four, a continuous collection sample loop was devised. The loop incorporated two sample locations placed roughly in the center of the treatment zones A and B (Figure X.1). Flexible 1/8" O.D. PEEK tubing Alltech Associates, Inc. (Deerfield, IL) was used for sample collection while, flexible 1/8" O.D. Teflon® tubing served as the return. Inlets were screened with No.40 stainless steel mesh to limit solids uptake. Water was pumped from a depth of 21'(below ground surface) by a Masterflex peristaltic pump to a continuous measurement flow cell at a rate of 10 ml/minute. The flow cell was fabricated from acrylic plastic with a segment of 1" O.D. tubing fastened to a ¼" thick plate. The open end of the tube was tapped for ½" nominal pipe threads (NPT) and two 1/8" NPT threads 4" on center were tapped along the vertical axis. Plastic 1/8" NPT to barbed nipples were used for flow connections to the plastic. The probe was sealed in the acrylic tube with a ½" O.D. x ½" NPT polypropylene compression fitting. The finished internal volume of the flow cell measured 17 ml. A Cole-Parmer® bromide combination glass body electrode (Vernon

Hills, IL) and an Accumett Model 25 pH/Ion meter, Fisher Scientific, (San Rafael, CA) system was used to measure free bromide in the samples. Data was continuously output from the meter through an RS-232C port to a Campbell Scientific Model 21X data logger (Logan, UT). Readings were averaged over five minute intervals and placed in final data storage. Programmed operation steps of the 21X data logger are shown in Appendix E. Water from the pump entered the cell at the base and flowed upward to an exit. Water exiting the cell was returned to the well by gravity. The cell was full-mixed by a Teflon coated stir-bar and magnetic plate assembly. Spacers below the flow cell allowed for air circulation and helped to minimize heat transfer from the stir plate. Temperature of the flow cell and surrounding ambient conditions were measured and recorded on five minute intervals by the 21X data logger. A detailed description of the continuous sample system is presented in Appendix D. The bromide probe was calibrated in standards created with potassium bromide and site groundwater. The site groundwater was deemed bromide free after six months of weekly anion measurements by ion chromatography.

The permeable reactor was installed without ceramic packing or cells and suspended over the screened interval of the well casing. A bromide solution of 10 grams/liter was injected at a flow rate of 1.16 liter/hour with an FMI QG-50 Fluid Metering Inc. (Oyster Bay, NY) positive displacement pump. The solution was injected in the anaerobic mixing zone for 60 minutes. De-ionized water was used to purge the bromide supply system. Bromide mixing was conducted by a gas lift system, which operated in a pulse mode. Nitrogen gas was incorporated in anaerobic zone while; oxygen gas was used in the aerobic zones. The liquid injection systems embedded in the aerobic zones were disabled. Mixing in the anaerobic and aerobic injection manifolds was automated with a timed electric solenoid valve Cole Parmer (Vernon Hills, IL) and occurred for 5 seconds every minute. Mixing time corresponded to the injection period and was terminated after 60 minutes. Data was automatically logged by the collector for the experiment, which lasted one week. Data was periodically transferred from the logger to a portable notebook computer. The elevation of water in the well casing was measured and referenced to mean sea level.

Tracer Test No. 7: Fall 1998

Installation of monitoring wells directly up gradient of the permeable barrier reactor in the late summer of 1998 provided an ideal location for the continuous injection of a conservative tracer solution. An FMI QG-50 Fluid Metering Inc. (Oyster Bay, NY) positive displacement pump was used to meter a concentrated solution of sodium bromide (100 g/L) to a 20-liter carboy. Fresh water was supplied as makeup to the carboy to provide a test solution that contained 100 mg/L of bromide. The volume of test solution was maintained in the carboy by an overflow weir type structure. The carboy was stirred continuously on a magnetic stir plate and had a residence time of 20 minutes. The movement of nitrogen gas through the bulk fluid also enhanced mixing. Nitrogen gas was dispensed through three sparging stones to strip dissolved oxygen from the test solution. The test solution was dispensed to the injection well (MW98-1) by gravity at a flow rate of 1 liter per minute.

Process samples were collected from several locations in and around the permeable barrier reactor over the 72 hour period of test solution injection. Samples were collected from reactor locations 7L and 7U using the gas lift sampling system. In addition, recirculating flow loops were used to collect continuous samples from the center of treatment zone 1 and 2 and from monitoring wells (98-1, 98-2, and 98-3). Figure X.1 displays sample locations relative to the in-situ treatment unit. A detailed description of cell A and B 1 location is found in Appendix D. Sample collection from the reactor system from points 7L and & 7U is outlined in Appendix H. A Cole-Parmer® bromide combination glass body electrode (Vernon Hills, IL) and an Accumett Model 25 pH/Ion meter, Fisher Scientific, (San Rafael, CA) system were used to measure free bromide in the samples. Authentic bromide standards were created in the laboratory and used to standardize the electrode on three-hour intervals.

RESULTS

Tracer Test No. 6

Bromide concentrations observed during the first five hours of the study are shown in Figure X.2. With time, bromide concentrations in the system steadily increased to approximately 35 mg/L. Following the observation of a uniform initial bromide concentration, injection of the tracer solution was terminated. Physical system constraints did not allow for traditional analysis of tracer breakthrough curves to estimate retention time. Therefore, the reactor system was evaluated as a continuous stir tank reactor and the disappearance of bromide from the system was monitored. Figure X.3 depicts bromide concentrations measured and predicted in the reactor system with time. A mass balance around the concentration of bromide was written. From the early response of bromide concentrations in the system, an initial concentration of 35 mg/L was chosen. Using concentration measurements with time, and the initial bromide value, the hydraulic retention time of the unit theta was computed for each time step. Values of theta in hours were tabulated and averaged over the duration of the experiment. Results of the individual time step calculations indicated an average theta of 27.9 hours. Summary statistics showed the standard deviation of the sample population was 5.35 hours. Using the calculated theta and initial conservative tracer concentrations observed, the response of bromide in the permeable barrier reactor was estimated. The computed values were then plotted against the observed field measurements (Figure X.3).

Tracer Test No. 7

Evaluation of bromide concentration with time and space allowed the generation of tracer breakthrough curves. Standard methods of data analysis were applied to compute the velocity of groundwater in the system. In the reactor, data collected from cell B was used to estimate the water velocity. Over the last seven hours of the experiment, the bromide concentration in cell B averaged approximately 72.4 mg/L. In the calculation of water velocity, the average value from cell B was assumed

to be the maximum concentration of bromide to arrive (C_{max}). The groundwater velocity was computed from the time that one half the concentration of C_{max} arrived at the sample location and the distance from the tracer injection point. Breakthrough curves of bromide concentrations measured in the permeable barrier system with time are presented in Figure X.4. From the figure, bromide concentrations measured in cell B approached one half of C_{max} (36.2 mg/L) at approximately 25 hours after injection commenced. Computation of the groundwater velocity from the distance from MW98-1 to cell B (94 cm) and the travel time resulted in an estimated groundwater velocity of 1.04×10^{-3} cm/sec. Applying the velocity of the groundwater to the linear distance of the permeable barrier reactor (61 cm) yields a residence time of 16.2 hours.

SUMMARY

Conservative tracer tests conducted in the field estimated the hydraulic residence time of the permeable barrier reactor at 27.9 and 16.2 hours. The tests were conducted under two completely different sets of physical assumptions yet; they each yielded similar results. In all likelihood, tracer test seven mostly accurately describes the conditions of flow in the reactor structure. It is difficult to compare the values of residence time to each other with a high degree of certainty. Results however do suggest that despite the presence of flouring biological community, groundwater flow through the unit was not significantly impacted after one year of system operation.

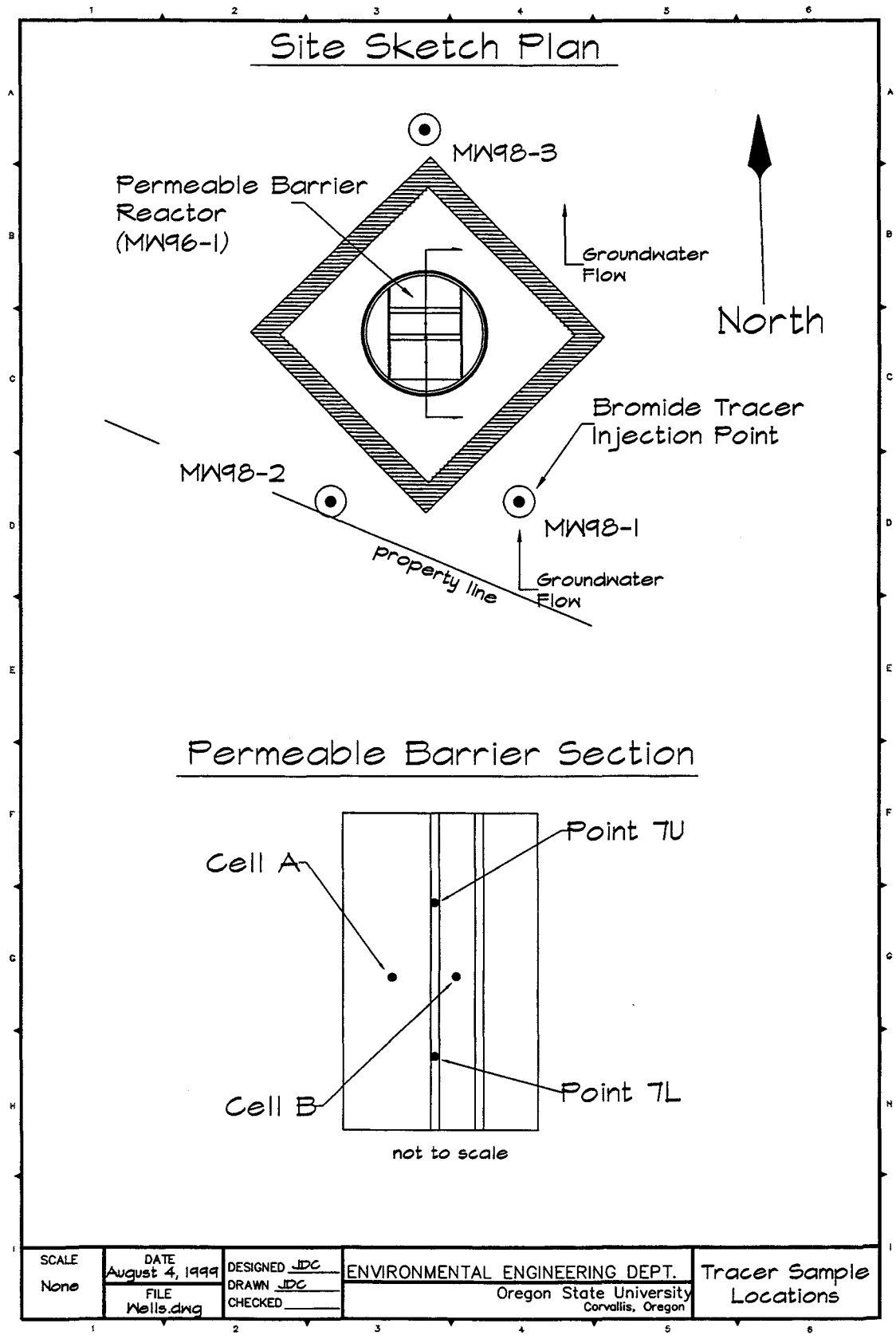

Figure X.1 Location plan of conservative tracer release and process sample points

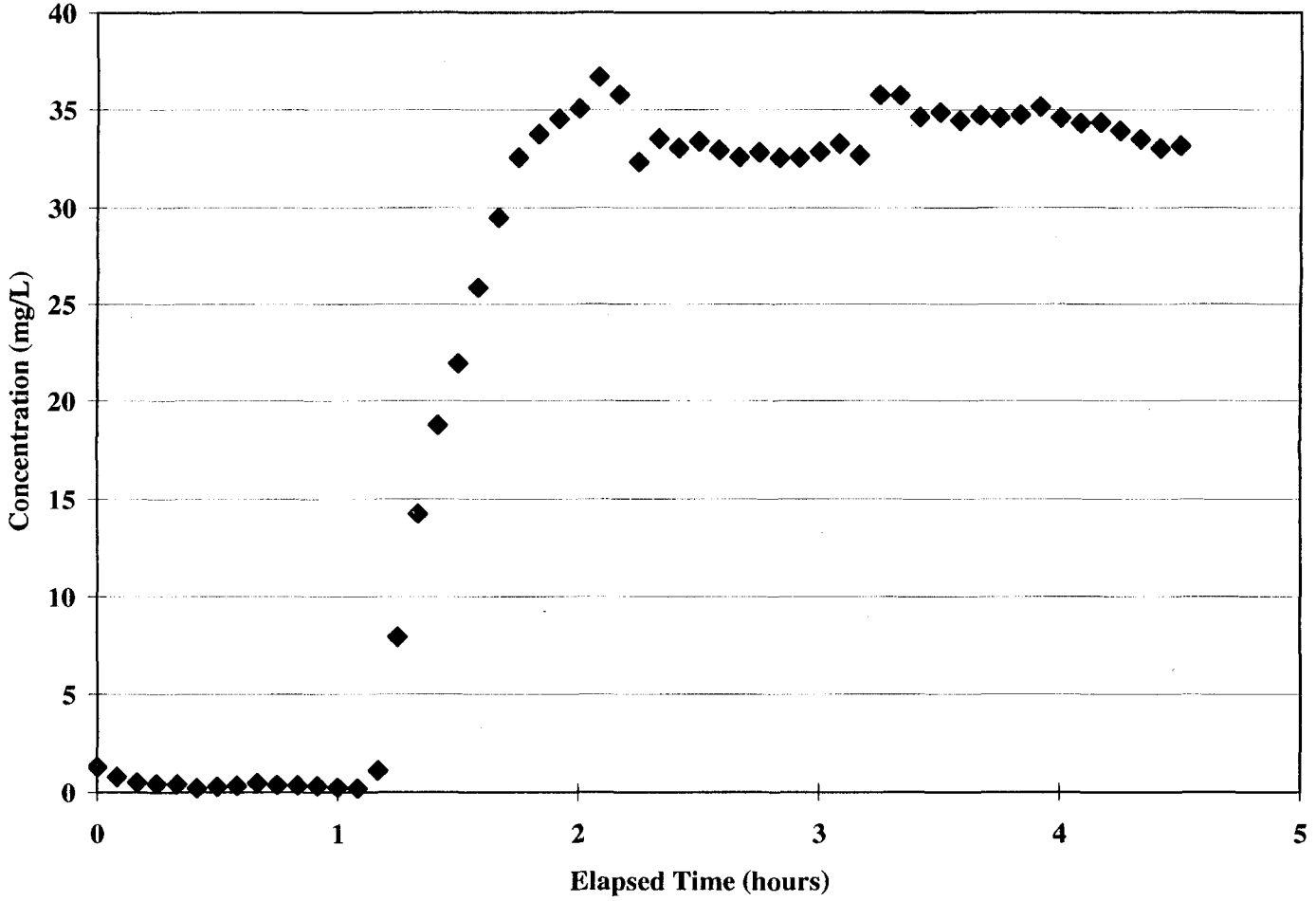

Figure X.2 Tracer six early time bromide concentrations

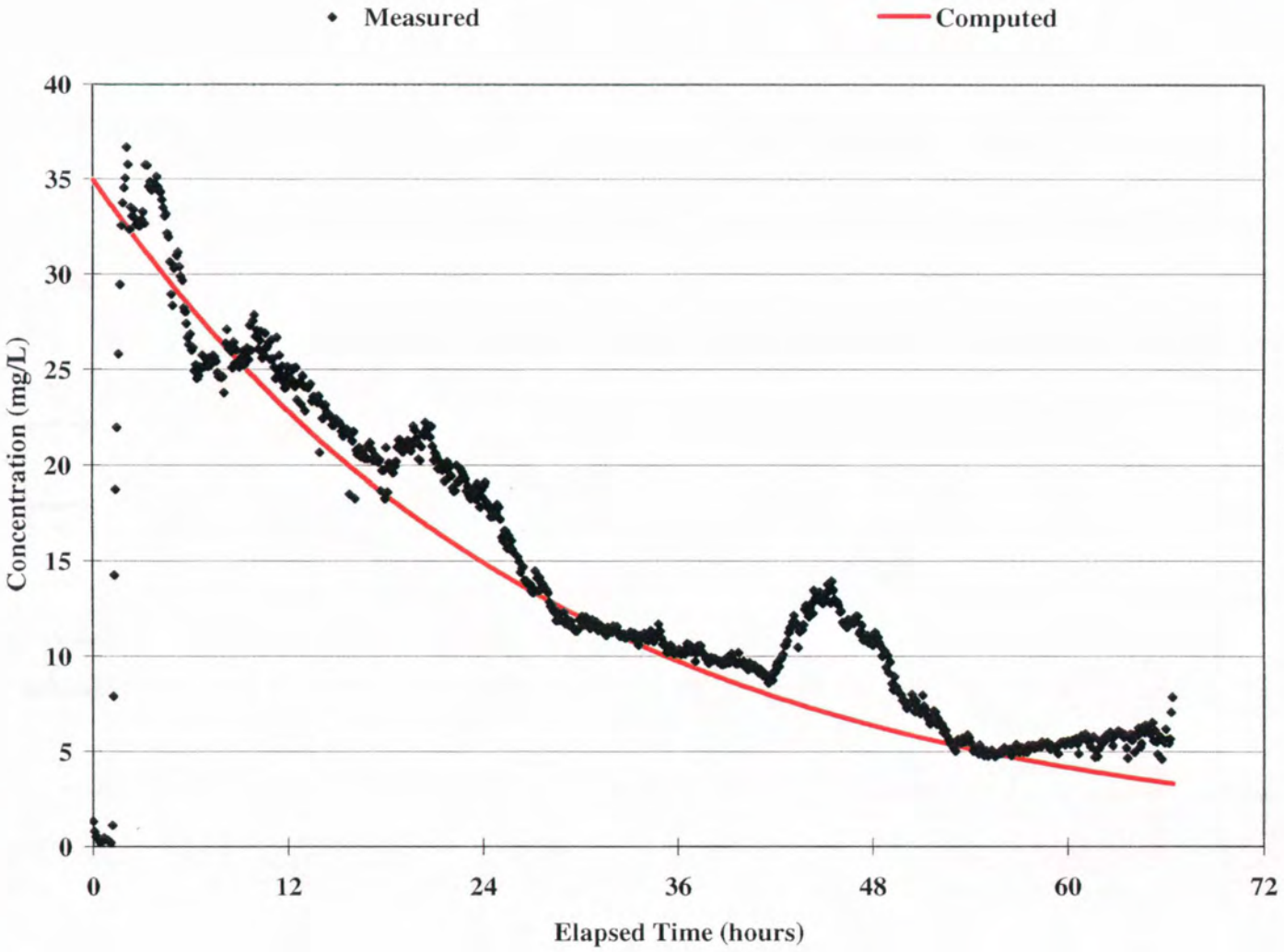

Figure X.3 Tracer six bromide response predicted and measured

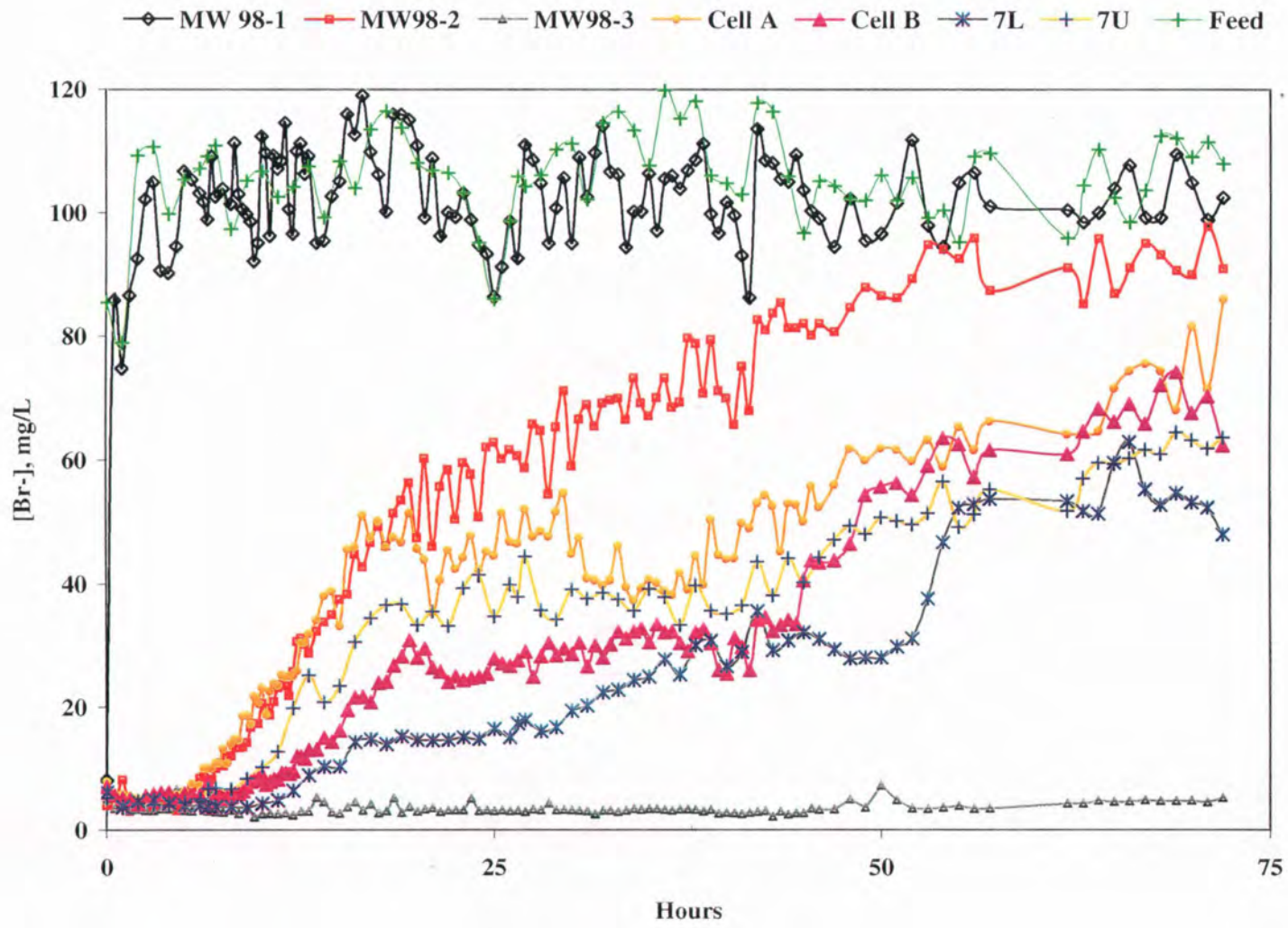

Figure X.4 Tracer seven bromide breakthrough curves