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Biotransformation of PCBs in Substation Soils

A Review of Laboratory and Pilot-Scale Testing for the Development of an In Situ Process for PCB

Technical Report

Biotransformation of PCBs in Substation Soils

A Review of Laboratory and Pilot Scale Testing for the Development of an in Situ Process for PCB Biotransformation in Soils

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REPORT SUMMARY

In situ methods are desirable for PCB remediation to prevent disruption of activities at industrial sites such as substations. This study follows the development, from laboratory testing through pilot-scale demonstration, of an in situ soil irrigation process for biotransformation of PCBs in soils.

Background
Widespread environmental PCB contamination is estimated to have occurred simply from the sheer abundance of PCBs manufactured and because of the persistent nature of PCBs. Various microorganisms have been identified with capacity to transform highly chlorinated PCB congeners to more benign forms. Both aerobic and anaerobic microbial activities are desirable for efficient and effective PCB destruction. This report describes development of a process to use microbial capabilities for remediation of PCBs in substation soils. Microbial capabilities are captured in a process that recovers PCBs from soils and optimizes conditions for aerobic and anaerobic degradative activity.

Objective
To determine potential for and limitations of PCB biotransformation in substation soils, to formulate and test at laboratory and pilot scale a process for PCB biotransformation in situ, and to decide the effectiveness of a field-scale application of the process.

Approach
Analytical capability for PCB identification and quantification was established at the PCB congener level. The project team constructed recombinant bacteria with the capabilities of metabolizing a nonionic surfactant as carbon source and degrading lower chlorinated PCB congeners. The surfactant was employed in a method to recover PCBs from soil, making them bioavailable. The team subjected PCBs to anaerobic dechlorination that resulted in the availability of lower chlorinated congeners for additional aerobic degradation. Results were collated to design a process. Unit processing steps were optimized at the laboratory scale and combined for a demonstration of the entire process at pilot scale under one roof. Pilot-scale results were used to predict the success of the process at field scale.

Results
A research site was selected and the scope of PCB contamination at the site characterized. The research site was four capacitor bank areas on the Moccasin Bend Substation, Chattanooga, Tennessee. Laboratory studies confirmed the lack of competent microbial populations in the substation soil to either aerobically degrade or anaerobically dechlorinate PCBs. Laboratory studies confirmed successful aerobic degradation and anaerobic dechlorination of PCBs when

the substation soil was bioaugmented. Information gathered was incorporated in a scheme for an in situ soil irrigation process to optimize PCB biotransformation of substation soil. The process combined components of PCB recovery, aerobic degradation, and anaerobic dechlorination. The efficiencies of unit processing steps were determined at both laboratory and pilot scale. The project identified opportunities for improving unit operations. By performing the process through two cycles of both aerobic degradation and anaerobic dechlorination of PCBs at pilot scale, the efficiency of the overall process was determined. The combination of unit processing steps at laboratory scale accounted for a calculated PCB biotransformation efficiency of 56%. The efficiency of the overall process under pilot-scale operating conditions was 21%. This relatively low pilot-scale efficiency signals low probability for a successful field deployment of the process as it is currently practiced.

EPRI Perspective

In situ technologies for destroying soil contaminants related to electric utility operations have potential to save operating costs. In situ technologies can prevent interruption of operations and can save costs of moving contaminated soil off site. Relatively low levels of contamination in soil, such as the approximate 100 ppm PCBs encountered for this study, demand the same requirements for remediation as higher levels of contamination. If levels of PCB contamination below 100 ppm are of concern, the in situ soil irrigation process for PCB biotransformation in soils may have potential to reduce PCB concentrations even further to acceptable levels. However, for relatively greater PCB soil concentrations, the pilot-scale results reported in this document predict a low potential for success in a field application of the process as it is currently practiced.

Keywords

Biotransformation
PCBs
In situ treatment
Substations

ABSTRACT

Polychlorinated biphenyls (PCBs) have been widely used in the electric utility industry because of their excellent heat transfer properties. However, polychlorinated biphenyl (PCB) manufacture in the United States was halted in 1979. The use and disposal of PCBs is highly regulated. Excavation and burial of contaminated soil have been the most accepted disposal means for PCB spilled on substation soils. Use of landfill space for PCB disposal is becoming more unacceptable. Incineration is generally unpopular to the public. Treating large quantities of low level PCB-contaminated soil by these same techniques may result in additional expenses due to interruption in normal operating activities. On-site PCB destruction in these soils is desirable.

An in situ soil irrigation process for PCB biotransformation in soils has been developed and tested at pilot scale. Soils with low levels of residual PCBs can benefit most from the process. Unit processing steps, developed at laboratory scale and demonstrated at pilot scale, include soil washing, aerobic degradation, liquid-solids separation, and anaerobic dechlorination. Laboratory scale development of the unit processing steps resulted in an overall process PCB destruction efficiency of 56%. Care was taken during the pilot scale demonstration of the process to track all reactants, products, and sampling operations for a mass balance. The PCB degradation efficiency during pilot scale biological operations was 42%. The soil wash cycle for recovering PCBs from soil prior to the biological operations was 50% efficient. Thus, the overall pilot scale PCB biotransformation efficiency was 21%.

The background for the process development is provided along with results of optimizing each process step and the outcome of process testing at both laboratory and pilot scale.

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are a family of compounds produced commercially by the batch chlorination of biphenyl. Resulting technical mixtures contain a given chlorine content as a consequence of the duration of the chlorination process (De Voogt et al. 1990). In theory, chlorine can be placed at any or all of the ten available sites on the biphenyl nucleus, and 209 polychlorinated biphenyl (PCB) molecular species, known as congeners, are possible. Of these possible congeners, only about half are actually produced during synthesis due to steric hindrance. PCB products vary from light oily fluids with di-, tri-, and tetrachlorobiphenyls to heavy oils with pentachlorobiphenyls to greases and waxes of highly chlorinated biphenyls.

In July 1979, the manufacture, importation, distribution, and further processing of PCBs in the United States was banned (Hooper 1990). Prior to this date, PCBs were manufactured and sold under various trade names. In the United States, most were sold as "Aroclor", produced by Monsanto. Other product names are Fenclor in Italy, Phenoclor and Pyralene in France, Clophene in Germany, and Kanechlor in Japan. The various PCB products are designated by overall chlorination, but each product contains a variety of congeners. For example, Aroclor 1248 is 48% chlorine by weight.

PCBs have desirable physical and chemical properties, such as low vapor pressure and water solubility, and excellent dielectric properties. They are stable to oxidation, flame resistant, and relatively inert. PCBs have been shown to accumulate in the lipids of many organisms due to their non-polar nature (Kimbrough 1974). They are suspected human carcinogens although occupational exposure has not been linked to cancer incidence (Kimbrough 1987, Kimbrough et al. 1975). PCBs have been implicated as playing a role in the hormonal disruptions that have resulted in decreased reproductive competence in animals and humans (Soto et al. 1995).

The properties that make PCBs desirable to industry are cause for their persistence in the environment. Environmental contamination is estimated to have occurred simply from the sheer abundance of PCBs manufactured (Pal et al. 1980) and the persistent nature of PCBs. Thus, industrial sites, such as electric utility substations, may have low levels of residual soil PCBs in spite of efforts to adhere to regulations for spill cleanup. Cost effective remedies are needed for soils containing low level contamination. In situ bioremediation technologies are cleanup methods worthy of investigation.

Aerobic Degradation of PCBs

Microbial mechanisms of PCB degradation have been reviewed by Furukawa (1982), Abramowicz (1990), Hooper et al. (1990), Unterman (1996), and Bedard and Quensen (1995). Degradation of the biphenyl ring has been shown to be an aerobic process. Dechlorination of highly-chlorinated congeners takes place in an anaerobic environment.

Aerobic bacterial degradation of PCBs typically proceeds via the oxidative biphenyl pathway encoded by the *bph* genes (Furukawa 1982, Lajoie et al. 1994). The initial step in the oxidative pathway for PCB degradation, the so-called upper PCB pathway, is the insertion of an oxygen molecule into the aromatic ring to form a chlorinated *cis*-dihydrodiol (Furukawa 1982). This 2, 3-dihydroxylation is catalyzed by a three-component biphenyl dioxygenase (BPH dox). The exact structure of this enzyme varies from one species of bacteria to another and determines the selectivity of the bacterium for various PCBs (Mondello et al. 1997). The second enzyme of the *bph* pathway is the dihydrodiol dehydrogenase (BphB). The third enzyme is 2, 3-dihydroxybiphenyl dioxygenase (BphC). The fourth enzyme is 2-hydroxy-6-oxo-6-phenylhexa-2, 4-dienoic acid hydrase (BphD). The compounds resulting from the upper PCB pathway are chlorinated benzoates and acetophenones. The degradation of the chlorinated benzoates constitute the so-called lower pathway. Few bacterial strains catalyze both the upper and lower pathways. A number of bacteria degrade chlorobenzoate to substrates for central metabolic pathways (Chatterjee and Chakrabarty 1981). The chlorinated benzoates can be further degraded aerobically (Arensdorf and Focht 1994) and anaerobically (Mohn and Tiedje 1992). Acetophenones can be further degraded, also (Havel and Reineke 1993).

Biphenyl-utilizing soil bacteria are widely distributed in the environment (Furukawa 1994). A number of aerobic microorganisms can degrade lightly chlorinated biphenyls. Furukawa et al. (1978a, 1978b, 1979) demonstrated that bacterial strains have markedly different activities against specific PCB congeners. Some microorganisms degrade an exceptional range of congeners (Barriault et al. 1998). However, aerobic degradation of PCB mixtures generally follows a pattern related to the position of the chlorines on the biphenyl rings. Degradation decreases as chlorine substitution increases. Congeners with chlorines on a single ring are degraded faster than those with two substituted rings. Congeners containing ortho position chlorines are poorly degraded. Congeners with two ortho chlorines are extremely resistant to degradation (Furukawa 1994). Congeners with lesser chlorinated rings are degraded prior to those with more highly chlorinated rings. In general, the position, not the number of chlorines, has a significant influence on degradation patterns (Furukawa et al. 1978, Furukawa et al. 1979). With few exceptions, bacteria do not use congeners as growth substrates.

Bedard et al. (1986) found three strains to be the best aerobic PCB-biodegrading bacteria among a number of bacteria tested. They compared degradation on 18 selected congeners, including quatra-, penta-, and hexachlorinated biphenyls. The strains were *Corynebacterium* MB1 (also referred to interchangeably as *Acinetobacter* sp. strain P6, *Arthrobacter* sp. strain M5, and *Rhodococcus globerulus* P6), *Alcaligenes eutrophus* H850, and *Pseudomonas* sp. LB400 (also referred to interchangeably as *Burkholderia cepacia* LB400). *Burkholderia cepacia* LB400 has been studied extensively because it attacks a variety of di- to pentachlorinated biphenyls (Bopp 1986, Haddock et al. 1995). *Burkholderia cepacia* LB400 and *Alcaligenes eutrophus* H850 can oxidize chlorobiphenyls at the 2, 3 and 3, 4 positions (Nadim et al. 1987). *Rhodococcus globerulus* P6, under its various names, has been studied extensively (Furukawa et al. 1978a, 1978b, and 1979). *Rhodococcus globerulus* P6 has been shown to contain *bph* genes unlike those of the gram negative species (Asturias and Timmis 1993, Asturias et al. 1994). Bacteria with the ability to mineralize biphenyl are widespread in soils and sediments (Wagner-Döbler et al. 1998).

Bedard and Haberl (1990) performed resting cell assays to determine the extent of congener depletion, route of metabolism, and degradation of intermediate metabolites by eight species of PCB-degrading bacteria. They grouped the bacteria on the basis of congener degradation. The two strains with the broadest range of congener attack were *A. eutrophus* H850 and *Pseudomonas* sp. LB400. Congener degradation patterns suggest that these two strains have both 2, 3 and 3, 4 dioxygenase activity. The strain *Corynebacterium* sp. MB1 alone demonstrated the unique property to attack the biphenyl ring having one chlorine, in spite of the chlorination pattern of the other ring. All bacteria tested metabolized poorly the congeners with 2, 4 chlorination. This trait is unfortunate because of the dominance of 2, 4' chlorination in environmental PCBs.

Gibson et al. (1993) highlighted the versatility of *B. cepacia* LB400 in comparison to the strain *Pseudomonas pseudoalcaligenes* KF707 (Furukawa et al. 1989). Although the two strains were shown to be similar by DNA-DNA hybridization, they presented differing abilities to oxidize PCB congeners. In addition, the differences were not related to congener transport specificities. Kohler et al. (1988) further explored the utility of *Acinetobacter* sp. P6 for Aroclor 1254 degradation and showed it to transform a greater PCB congener range than is reported for *A. eutrophus* H850.

Furukawa (1982) reviewed microbial PCB metabolism in aquatic environments. In addition, there are many literature citations concerning the performance of PCB-degrading organisms in liquid culture media. There are few reports of PCB degradation in soil (Barriault and Sylvestre 1993, Brunner et al. 1985, McDermott et al. 1989, Viney and Bewley 1990).

Most studies of PCB biodegradation in soil are based on the addition of fresh congeners, congener mixes, or Aroclors to soil. There are limited accounts of biodegradation of weathered PCBs in soil. Unterman et al. (1985) used sand as a model to demonstrate that PCBs were biodegradable when bound to a solid substrate. Subsequent studies are slanted toward addition of specific bacterial strains to soils verified to have biphenyl-metabolizing capacity. Viney and Bewley (1990) demonstrated that slow Aroclor 1242 mineralization occurs in natural soils. The turnover time for PCB mineralization in sewage sludge was estimated to be from 6 to 26 years, with 95% removal of 500 mg/kg Aroclor 1242.

Fiebig et al. (1993) showed that improved PCB degradation can result from nutrient stimulation of bacteria. However, biphenyl addition is a more important factor for improved PCB degradation. It is practical to add biphenyl for bioremediation because biphenyl has approved use as a post-harvest fungicide for prevention of molds on fruit, particularly citrus, and for impregnation of packaging materials (Kramer 1983). The LD_{50} by oral dose in rats is 3280 mg/kg. Although biphenyl is insoluble in water (Weast 1984), it can be applied to soil surfaces dissolved in such solvents as hexane or acetone using an atomizer (Hickey et al. 1993).

Several researchers have shown that biphenyl is necessary for aerobic PCB degradation (Adriaens et al. 1989; Barriault and Sylvestre 1993; Brunner et al. 1985; Focht and Brunner 1985; Kohler et al. 1988). Kohler et al. (1988) demonstrated the importance of biphenyl for liquid culture PCB cometabolism. They reported that Aroclor 1254 was transformed as biphenyl disappeared from culture. Biotransformation ceased after biphenyl depletion. Focht and Brunner

(1985) and Brunner et al. (1985) further showed that enrichment with biphenyl was the most important factor in promoting degradation of a chlorinated biphenyl mixture. Biphenyl enrichment improved soil PCB biodegradation. To improve degradation, *Acinetobacter* P6 was introduced as a PCB cometabolizer. Its presence produced additional enhancement beyond that of the natural microflora. However, biphenyl addition was the more significant degradation effector in comparison to the added bacterial strain. Repeated biphenyl addition further improved degradation.

Barriault and Sylvestre (1993) further verified the importance of biphenyl addition showing the value of repeated biphenyl additions to microcosms receiving a PCB degrader inoculation. Repeated biphenyl additions maintained a relatively high biphenyl concentration over the incubation period. Repeated biphenyl additions did not improve the PCB degradation rate, however. Degradation rate was actually greatest after the initial biphenyl addition. Degradation rates diminished as each subsequent biphenyl addition was made.

Mondello (1989) produced a recombinant *Escherichia coli* strain containing the biphenyl degradation pathway which could degrade selected congeners without growth on biphenyl. However, his work left room for speculation that the improved degradation, in comparison to that of parent *Pseudomonas* sp. LB400, was due to increased copy number.

It is well documented that addition of known degrading bacterial strains to soil improves PCB degradation in comparison to degradation by native populations (Barriault and Sylvestre 1993, Furukawa et al. 1989, Hickey et al. 1993, Unterman et al. 1985). Bedard et al. (1987) suggested that the activities of various bacterial strains may complement each other for the most complete PCB degradation that can be achieved. They tested Aroclor 1254 degradation by each of two PCB degraders alone and projected an improved degradative capability from the combination *Corynebacterium* MB1 and *A. eutrophus* H850. Unterman et al. (1985) demonstrated the additive effect of the two strains in soil.

Barriault and Sylvestre (1993) stated that efficient PCB degradation can only be achieved when all known catalytic activities are present in a given medium. These activities may have to be supplied by addition of known degraders to soil. Survival of these introduced organisms is critical. Improved survival was achieved by Barriault and Sylvestre (1993) through repeated soil inoculation with a PCB degrading *Ps. testosteroni*.

Hickey et al. (1993) further explored the benefits of adding known degraders to soil for PCB mineralization. They added either a chlorobiphenyl or a chlorobenzoate degrader and combinations of the two along with biphenyl to labeled Aroclor 1242 spiked soil. Their contention was that chlorobenzoate inhibits bacterial PCB metabolism; its removal may improve PCB degradation. The chlorobenzoate-degrading strain *Ps. aeruginosa* alone, rather than in combination with the chlorobiphenyl degrader or in comparison to the chlorobiphenyl degrader, provided the highest PCB disappearance rates and mineralization levels in the soil. They concluded that chlorobenzoate metabolism in soils is a limiting factor in Aroclor 1242 mineralization by native populations. Destruction of chlorobenzoate metabolites such as chlorocatechols probably eliminates inhibition problems. Sondossi et al. (1992) demonstrated degradation metabolite inhibition by mixed populations. They found that the more readily

degradable chlorinated benzoates accumulated the more potent inhibitors, producing a self-defeating situation for PCB degradation. Thus, the improvements Hickey et al. (1993) found in PCB degradation with chlorobenzoate degraders may have two explanations. Improvement may be due to chlorobenzoate degradation or genetic exchange which rendered the chlorobenzoate degrader capable of chlorobiphenyl degradation. Whatever the case may be, degradation improves with microbial consortia having multiple capabilities.

Focht and Brunner (1985) demonstrated the importance of a high initial population density when supplementing soil inoculated with active degraders. To overcome the difficulty in correlating the number of biphenyl oxidizers to biphenyl degradation rate in soil, they determined biphenyl degradation by measuring labeled carbon dioxide released from labeled biphenyl. Breakdown of the biphenyl was not assurance of PCB mineralization since complete mineralization of chlorinated biphenyls requires the presence of genetic capability to not only attack the chlorinated biphenyls but to further degrade the typically resulting chlorobenzoic acids (Sondossi et al. 1992). Focht and Brunner (1985) learned that without additional populations in soil, application of a degrader species is ineffective. Thus natural consortia appear necessary for effective bioaugmentation in soil.

In preparation for a soil field test, Unterman et al. (1985) grew large volumes of *Pseudomonas* sp. LB 400 using biphenyl as sole carbon source. Inoculum preparation on biphenyl was performed in lieu of adding biphenyl to the soil. They tested parameters such as frequency of adding bacteria to soil, mixing of soil, temperature effects, and medium effects. They found that adding debris from killed cells actually stimulated a low level of indigenous PCB degrading capacity in the soil. They documented the need for repeated applications of active bacteria to soil for PCB degradation. The findings of Kohler et al. (1988) support the need for additions of bacterial cultures. They demonstrated that growing, rather than resting, *Acinetobacter* sp. P6 and *Arthrobacter* sp. B1B cells transformed a wider range of congeners.

Anaerobic Dechlorination

Reductive dehalogenation is necessary for biodegradation of highly chlorinated PCBs (Mohn 1992). Reductive PCB dechlorination by anaerobic microorganisms has been demonstrated in the laboratory and in situ (Abramowicz 1990; Alder et al. 1993; Brown et al., 1987a, 1987b; Quensen et al. 1988). Microbial PCB dechlorination was discovered when it became apparent that PCBs in several aquatic sediments had undergone transformation, mainly removal of meta and para chlorines (Bedard and Van Dort 1997). Other reports have confirmed the anaerobic ability. Recently, dechlorination of ortho chlorines in a commercial PCB mixture has been demonstrated (Wu et al. 1998). Alterations of PCBs in Hudson River anaerobic environments indicate microbial degradation of highly chlorinated biphenyls (Brown et al. 1984, Brown 1987a and 1987b). PCB dechlorination in the various environments follows patterns that suggest discrete populations of PCB-dechlorinating microorganisms in the environments (Wu et al. 1997). In addition, Stevens et al. (1988) identified an anaerobic sulfidogenic strain capable of reductive dichlorobenzoate dechlorination.

Anaerobic microbial consortia capable of PCB dechlorination leave the biphenyl nucleus untouched, resulting in lesser chlorinated congeners. No anaerobic microorganisms that dechlorinate PCBs have been isolated, cultured, or characterized. Sediment or soil is required for successful transfer of dechlorinating activity in culture (Wu and Wiegel 1997). Although recently rather specific dechlorinating activity has been maintained in repeated culture using sediment free defined media (Cutter et al. 1998, Wu et al. 2000). Dechlorinating activity can be serially transferred from culture to culture of sediment (Morris et al. 1992). The activity is believed to result from enzymatic reactions carried out by several different strains or species of PCB-dechlorinating bacteria (Bedard and Quensen 1995).

The bioremediation process with greatest potential for degradation of highly chlorinated PCBs to lesser chlorinated compounds and mineralization of the intermediates consists of both aerobic and anaerobic activity (Bedard et al. 1986, 1987). Aerobic and anaerobic processes do not go on in the same region in nature, but can occur if processes are partitioned. A route which connects the two microenvironments is an apparent need for the most successful PCB biological degradation in soil. Bedard and Haberl (1990) proposed an anaerobic dechlorination strategy as a pretreatment to aerobic degradation.

White Rot Fungus

The white rot fungus, *Phanerochaete chrysosporium*, has potential for the degradation of PCBs (Bumpus et al. 1985, Yadav et al. 1995) although the extent of degradation effectiveness is in question (Dietrich et al. 1995). Mechanisms and pathways for PCB degradation are not detailed as with the aerobic bacteria. Bumpus et al. (1987) reported that mineralization of Aroclor mixtures proceeded more rapidly than mineralization of the single congener, ^{14}C-2, 4, 5, 2', 4', 5'-hexachlorobiphenyl, because of a synergistic degradation effect in a complex congener mix. Thomas et al. (1992) monitored degradation of labeled biphenyl and PCBs concluding that there is no direct correlation between degradation and production of fungal ligninases. They concluded that ligninases are not directly involved in PCB metabolism. PCB breakdown was associated with secondary phase growth rather than exponential growth.

White rot and other fungi are unaffected by biphenyl addition and produce nonspecific enzymes that enhance PCB transformation (Bumpus et al. 1987). Lamar and others (Anonymous 1993) found no effect of biphenyl concentration on the rate of its degradation, thus implying enzyme activity is not growth dependent. There were varied effects of manganese for enhancing ligninase on the biphenyl degradation rate. Enzyme production could not be correlated with biphenyl degradation. Chlorinated biphenyl disappeared during incubations due to its binding to fungal mats. Binding increased with increasing congener chlorination. Difficulty was experienced in obtaining mass balances, and labeled chlorinated biphenyl was found in connecting tubing. Various types of laboratory tubing materials show a range of PCB adsorption and desorption values (Cseh et al. 1989) Teflon is the best candidate for use in biological laboratory work with PCBs. Many laboratory materials adsorb PCBs leading to possible erroneous conclusions about the PCB disappearance due to biodegradation. The conclusions of Lamar and others suggest that disappearance of PCBs due to incubation with *Ph. chrysosporium* may be due to adsorption rather than PCB degradation.

Bioavailability

Microbial competency limits the effectiveness of PCB degradation or dechlorination. Bioavailability is another limiting factor. Physical and chemical characteristics of PCBs, including solubility, distribution between air and water, and sorption to soil, influence the extent of contact between PCBs and competent PCB-degrading microorganisms. Of these, PCB sorption to soil has the greatest influence on rate and extent of biodegradation. There is increasing PCB sorption in soils as the soil organic content increases (Cseh et al. 1989). The amount of PCB desorbed and available to microorganisms is related to congener water solubility. There is a trend toward increased congener biodegradability with increasing congener water solubility (Barriault and Sylvestre 1993). Lower molecular weight congeners are generally more soluble than higher molecular weight congeners.

Surfactant addition is one means to overcome some physical limitations of solubility (Electric Power Research Institute 1985). Contaminants may be strongly adsorbed to soil surfaces or trapped in soil pores. Surfactants can increase the solubility of hydrophobic compounds enhancing the apparent solubility of contaminants such as hydrocarbons and, therefore, also their desorption from soils.

Surfactants are amphiphilic and comprised of molecules with a soluble and an insoluble moiety. Micelles, organized surfactant structures, form spontaneously when surfactant concentration is above a level referred to as the critical micelle concentration (CMC). The CMC is the aqueous concentration of the surfactant at which surface tension is the smallest. Below the CMC, surfactant molecules exist solely as monomers. Above the CMC, surfactants orient themselves in aggregates so that, in aqueous solution, the polar portions of the molecules are pointed towards the bulk solution and the non-polar tails are oriented away from the bulk solution. The organic interior core of the micelle sequesters bulk solution. Thus, the micelles have a high capacity for solubilizing an organic contaminant and increase the overall carrying capacity of the fluid phase (Tadros 1984). In an aqueous system, the extent to which a solute will concentrate in a micelle can be related to the octanol-water partition coefficient (K_{ow}) of the solute. The larger the K_{ow} of a solute the greater will be its tendency to concentrate inside the micelle.

Nonionic surfactants are widely used to enhance wetting or detergency while producing little or no foam even under conditions where much mixing energy is applied. Alcohol ethoxylates offer the best overall compromise of low foam, low toxicity, and biodegradability among surfactants. Compared with cationic and anionic surfactants, nonionic surfactants show less antibacterial activity, and many are susceptible to microbial degradation.

Edwards et al. (1994) determined that the amount of surfactant required to solubilize the hydrocarbon, phenanthrene, in the presence of soil is considerably greater than the corresponding clean water CMC. They concluded that the difference between the surfactant dose necessary to attain the CMC in the soil-water system and a clean water system represents the amount of surfactant sorbed onto soil. In testing a combination of phenanthrene-contaminated soils and surfactants, they found the optimum surfactant dose in a 7:1 water to soil mix to be 1.5 mM/L surfactant. West and Harwell (1992) report the CMC typically ranges from 0.1 to 10 mM hydrocarbon.

Liu et al. (1995) reviewed the literature concerning effects of surfactant addition on microbial degradation of various hydrocarbons. Effects varied all the way from enhancement to inhibition

of degradation. They conclude that a variety of factors likely influence whether the addition of a particular surfactant enhances hydrocarbon recovery at the expense of inhibiting subsequent microbial degradation. Billingsley et al. (1999) recently reported the effects of several surfactants on both solubilization and degradation by *Pseudomonas* LB400 of various PCB congeners. They noted an increased rate of congener transformation with anionic surfactant and decreased rate with nonionic surfactants above the respective CMC of each. They suggested that the concentration of a surfactant solution with recovered PCBs should be diluted below the CMC prior to microbial treatment in order to optimize transformation. Tests by Fava and Di Gioia (1998) found the nonionic surfactant, Triton X-100, at a concentration above its CMC to negatively influence PCB depletion from soil in a 1:10 soil to liquid slurry. They provided proof that the effect was due to inhibition of bacterial population growth. On the other hand, in their fixed-phase reactors with a soil to liquid ration of 1:0.5, the surfactant had an enhancing effect. They concluded that the surfactant may make soil PCBs available, improving PCB degradation, yet inhibit PCB degradation where surfactant is readily available to the bacteria.

The value of surfactant in any bioremediation process must be carefully weighed because surfactant solubilization of the target compound may make it more bioavailable or move it off site. Surfactant may restrict PCB biodegradation efficiency due to the biological oxygen demand of the surfactant itself (Leavitt et al. 1992).

Barriault and Sylvestre (1993) showed the value of adding a bacterial stain that produces a surface-active agent along with a PCB-degrading organism. However, the improvement from surfactant-producing strain addition was not as great as the improvement that resulted from repeated biphenyl addition.

Viney and Bewley (1990) established a surfactant extraction scheme using 10 ml of surfactant solution per 2 g of PCB-spiked soil. They found that the surfactant solution improved extraction over the concentration range from 0.1 to 1 g surfactant per 100 ml distilled water. However, no further PCB release occurred above a surfactant concentration of 1g/100 ml. In addition, they found a range of tolerances to surfactants among PCB-degrading bacterial strains and *Phanerochaete chrysosporium*. They concluded that although a surfactant such as Triton X-100 is efficient at solubilizing PCBs, its inhibitory effects towards PCB-degrading organisms precludes it use in degradation strategies.

There have been few demonstrations of PCB bioremediation in soils. General Electric (GE) performed land treatment at a drag strip contaminated with weathered Aroclor 1248 (McDermott et al. 1989 and Unterman et al. 1985). A 50% reduction of total PCBs in the top centimeter of shallow soil was achieved without soil mixing. By comparison, soil mixing resulted in more widely distributed PCB degradation activity at soil depths and a 35% PCB reduction. GE conducted a bioslurry field study in the Hudson river showing that indigenous aerobic microorganisms degraded PCBs in anaerobically dechlorinated sediments (Harkness et al. 1990). The addition of *Alcaligenes eutrophus* H850 did not improve activity. However, degradation was stimulated by nutrients, biphenyl, and oxygen resulting in a 37-55% loss of the starting 39 ppm PCBs. Sorption of PCBs to the sediment was thought to be the reason greater degradation was not achieved. GE conducted an anaerobic PCB dechlorination in Wood Pond within the Housatonic River demonstrating 70, 57, and 27% dechlorination of respective hexa-, hepta, and octachlorobiphenyls and increasing tri- and tetrachlorobiphenyls. The Madison Metropolitan Sewerage District (Wisconsin) applied municipal sludge containing 50 ppm PCBs with an 85%

degradation of lower-chlorinated congeners and only 6% loss of a relatively recalcitrant marker congener (Gan and Berthouex 1994).

A bioavailability issue for anaerobic dechlorination relates to the PCB concentration on sediment. Researchers have shown direct relationships of concentration on sediment to both rate and extent of PCB degradation (Bedard and Quensen 1995). A minimum threshold value for dechlorination has been debated as well as a maximum value beyond which there is no dechlorination. The rate of dechlorination appears to show a linear response to PCB concentration on sediment over the range of about 100 to 1000 ppm PCB on sediment.

Biphenyl Addition

A number of reports verify that that biphenyl addition is a necessary factor for PCB transformation. Apparently microbes readily metabolize biphenyl but not chlorinated biphenyls. The necessity of biphenyl supports the hypothesis that cometabolism is the mechanism of PCB biotransformation (Adriaens et al. 1989, Barriault and Sylvestre 1993, Brunner et al. 1985, Focht and Brunner 1985, Kohler et al. 1988).

Although Bedard et al. (1987) found that specific bacterial cultures do grow on certain chlorinated biphenyls as carbon sources, as a general rule, biphenyl is required because cometabolism is the mechanism of PCB degradation. Recombinant bacteria dubbed field application vectors (FAVs) do not require the presence of biphenyl for PCB transformation (Lajoie et al. 1993). Development of FAVs is a method for expressing non-adaptive genes in a competitive environment. The method involves the use of a selective substrate, a host, and a cloning vector. In the application described herein, the FAV is based on surfactant-degrading bacterial strains containing either plasmid- or transposon-based, PCB-degradative genes. A constitutive promoter uncouples need for inducer from PCB cometabolism. Through the application of specialized FAVs in this integrated process, several advantages are achieved. The surfactant that is employed for soil washing to recover PCBs is subsequently degraded by the host strain. Additionally, the host strain degrades susceptible PCB congeners (Lajoie, et al. 1994).

Project Implications

This project seeks to develop a bioremediation approach for substation soils contaminated with a low level of PCBs. The widespread use of PCBs in the past may have led to just such low level contamination of substation and other industrial site soils. Sites of this type are generally isolated from any traffic and secured from access because of their use in power distribution and other activities. The electric utility industry takes reactive measures to rid the environment of PCBs. When past practice has indicated strong suspicion of PCB contamination, steps have been taken to conduct site assessments. Where warranted, contaminated equipment, soil, and the like have been subjected to standard treatments for PCB destruction. This project provides for a proactive program to get rid of PCBs in the environment. To accomplish this objective, the project consists of selection of a site with low level PCB contamination; site assessment; laboratory support to evaluate the potential of microbial, chemical, and physical activity for PCB degradation; a pilot scale trial to demonstrate combined physical/chemical/biological practices which reduce PCB levels; a feasibility assessment of the process; and research site closure.

2
LABORATORY STUDIES FOR DEVELOPING A BIOTRANSFORMATION PROCESS

The objective of the project is an in situ process for PCB destruction in substation soils. A variety of laboratory and field studies supported process development and led to a pilot scale trial of the process. The activities dealt with means to stimulate biological degradation of PCBs and to improve the availability of PCBs to biological attack.

Research Site Selection

A set of simple criteria were established to aid in selecting a workable research site. These included such criteria as a PCB soil concentration of less than 1000 ppm, a discrete area of contamination, the absence of other organic and inorganic contaminants, avoidance of drainage problems, and availability of past land use records and site history. Candidate sites were visually inspected and staff were interviewed for their knowledge of past practices. The inspections and interviews improved the chances of finding a research site with low-level residual PCBs in the soil and of holding down exploratory sampling and analysis costs.

Moccasin Bend Substation in Chattanooga, Tennessee, was selected. At the time of selection, the substation included four fenced capacitor banks with racks of PCB-containing capacitors. Records of all PCB spills and subsequent soil excavations were provided for project use. The substation had been in constant use for over 40 years. The capacitor banks had been in use for over 20 years. Substation records revealed that capacitors used at the substation had originally contained Aroclor 1242. The original construction plans; blue prints; a record of all spills, ruptures, and cleanups; and methods of weed control were provided for inspection. During the course of the project the capacitor banks were de-energized, and the capacitors and racks were removed. This allowed easy access to the contaminated soil. The cessation of herbicide weed control of the banks was requested. Over the course of the study, weeds were cut or pulled up by hand. The capacitor banks were locked so that access to the area was limited during the project life. The various record are archived at the TVA facilities in Muscle Shoals, AL.

After some initial exploratory sampling, it was found that PCBs were concentrated at the interface of the gravel and soil at the site. Throughout the report this specific soil layer, which is a mix of finely divided gravel and soil, is designated “interface”. Figure 2-1 is a schematic of a core through the gravel, interface, and soil at the substation showing the approximate depths and dimensions of the interface layer.

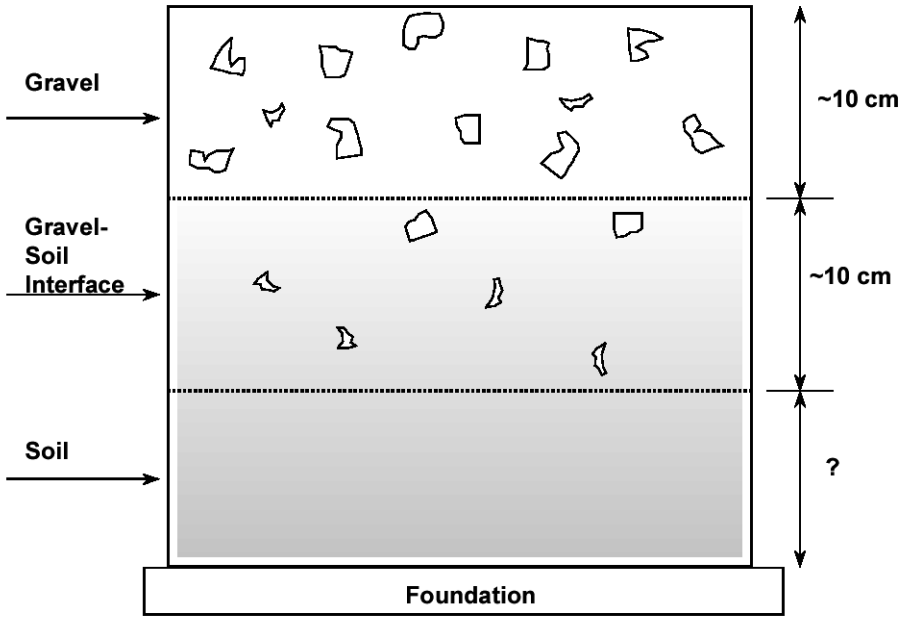

Figure 2-1
Schematic of Substation Core Sample with Depths and Dimensions of Gravel, Interface, and Soil Layers.

Gravel, interface, and soil were collected for laboratory and pilot tests on several occasions over the project's course. At each collection, bathroom scales were used to determine the tare weight of sterile, stainless steel storage containers and lids. Final weighings were made of filled and capped containers of gravel, interface, and soil. A chain of custody record was maintained on each soil and gravel batch. Representative subsamples from various containers were archived. Batches of gravel, interface, and soil were stored at 4º C. Archived samples were stored in a -20º C freezer.

Sampling and Analysis

A sampling and analysis plan was instituted for the four capacitor banks. The analytical methods and standards used to characterize the interface were described and references for these were documented (Beck et al. 1992). Figure 2-2 is a diagram of the sampling grid pattern. The points at which discrete samples were taken are indicated. Discrete samples were composited within seven sectors as indicated in the diagram. During site selection, PCBs were analyzed by Mid-South Testing Laboratory, Decatur, Alabama, following Method 8081. Thereafter, PCBs were analyzed by the peak identification method described in a succeeding section of this report. Additional soil properties were determined as described in the sampling plan. Microbial populations were enumerated using a most probable number method described more fully in a later section of this report.

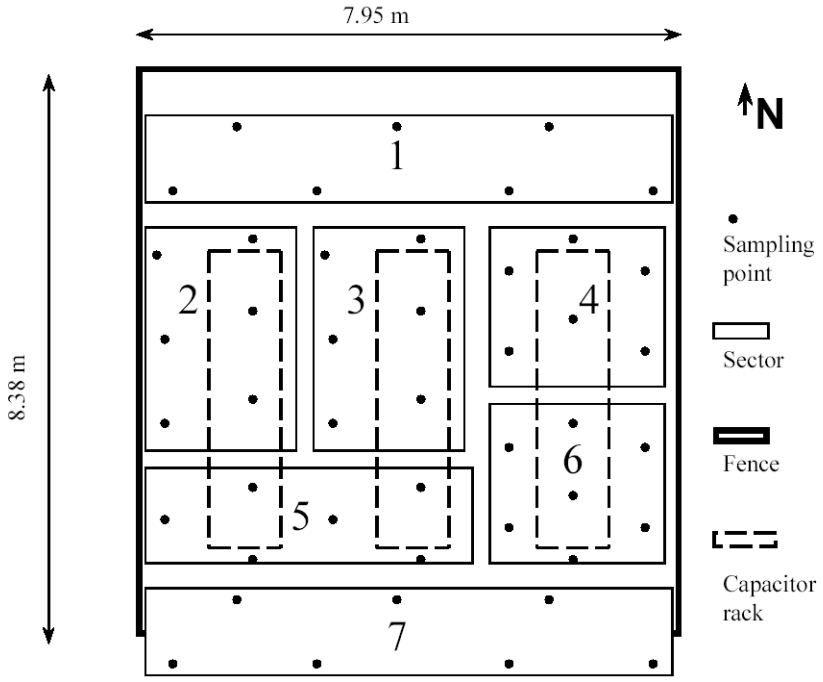

Figure 2-2
The Sampling Grid applied to each of the Four Capacitor Bank Areas consisted of Seven Numbered Sectors. Discrete Samples of Gravel, Interface, or Soil were taken at Sampling Points within a Sector. The Discrete Samples were mixed to present a Composite Sector Sample. Control Gravel, Interface, and Soil were Obtained on the Substation Property at a Distance of Greater than 15 m from the Capacitor Banks.

State and federal agencies were kept apprised of the disposition of soil and gravel samples throughout the project's course. Gravel, interface, and soil were provided to the Center for Environmental Biotechnology at the University of Tennessee (UT), Oak Ridge National Laboratory (ORNL), specified service laboratories for analysis, and to project staff at the TVA facilities in Muscle Shoals, AL, for experimentation. Throughout the life of the project, ongoing dialog was maintained with the Environmental Protection Agency (EPA), Region 4 and Headquarters, so that research using PCB-contaminated soil and gravel was conducted with approval necessary for handling specified quantities of PCBs. EPA views research and development for PCB destruction as equivalent to disposal. Removal, transport, and disposition of PCB-contaminated soil and gravel for laboratory and pilot scale testing were approved by Region 4. The Alabama Department of Environmental Management (ADEM) was notified about the disposition of soil and gravel at the TVA facilities in Muscle Shoals, AL. Records are archived in Muscle Shoals. A good deal of correspondence was conducted with Headquarters concerning the intent to conduct a field demonstration. This information which includes such details as containment measures, history of the recombinant bacteria, and means to adhere to health and safety requirements is archived in Muscle Shoals.

Research Site Characteristics

Table 2-1 contains the results of PCB analyses of the four capacitor banks. The relatively light PCB contamination met the site selection criteria of PCB contamination at less than 1000 ppm PCBs. The identification of the PCBs as Aroclor 1248, reported by the testing laboratory, was confirmed using the analytical protocol described herein providing peak identification. Table 2-2 contains the analysis of a core sample at 5 cm incremental depths in bank 3. The data suggest that there is negligible downward migration of PCBs in the substation soil.

Table 2-1
PCB Concentration in Four Capacitor Banks

Capacitor Bank	Sector	Gravel (ppm PCB)	Interface (ppm PCB)	Soil (ppm PCB)
1	1	1.1	No data	0.6
	2	39.4	No data	11.2
	3	26.4	No data	59.6
	4	15.7	No data	4.8
	5	29.9	No data	15.8
	6	7.8	No data	7.6
	7	0.6	No data	0.3
2	1	9.4	No data	3.9
	2	18.6	No data	13.4
	3	31.1	No data	10.6
	4	39.4	No data	12.6
	5	1.5	No data	2.7
	6	4.1	No data	13.5
	7	0	No data	0.3
3	1	0.5	4.0	0.6
	2	1.7	111.9	1.9
	3	1.1	40.7	1.7
	4	9.9	41.6	1.7
	5	28.9	137.6	9.1
	6	0	136.5	4.2
	7	9.4	17.3	2.6
4	1	3.5	7.2	0.7
	2	76.6	13.2	1.7
	3	8.5	38.6	10.9
	4	4.9	25.1	1.6
	5	47.8	33.7	9.5
	6	3.0	36.9	4.2
	7	2.5	20.1	0.8

Table 2-2
Results of a Core Sampling of Capacitor Bank Number 3

Sampling Core Depth	PCB (ppm Aroclor 1248)	Sample Description
0-5 cm	90.980	Gravel
5-10 cm	81.300	Interface
10-15 cm	43.400	Interface/Soil
15-20 cm	1.196	Soil
20-25 cm	2.094	Soil
25-30 cm	3.509	Soil
30-35 cm	4.526	Soil

The substation interface layer consists of a mix of particles from about 3 cm in diameter to finely ground limestone rock and sandy-clay chert. Characteristics of capacitor bank 3 interface are given in Table 2-3. Concentrations of the various metals tested, with the exception of calcium, are below or within normal ranges of typical agricultural soils (Alloway 1990). The relatively low action exchange capacity favors availability of the micronutrients for microbial growth and metabolism. The micronutrient profile resembles a typical agricultural soil. However, no phosphorus was found in the capacitor bank strata. The lack of phosphorus probably limits microbial growth as does the low level of nitrogen. Organic compounds, with the exceptions of PCBs and xylenes, were below detection levels. Xylenes were only slightly above the detection limit. No pesticides were found although a commercial mix of trifluralin and tebuthiuron was used at Moccasin Bend for weed control. Most of the total carbon can be accounted for as calcium carbonate, the composition of the limestone gravel cover. The balance of carbon, presumed to be organic carbon, is relatively low in concentration. It was concluded that there are no inhibitory factors precluding either the stimulation of native microbial populations or the survival of added exogenous PCB-degrading strains.

The microbial population of the control soil was compared to populations of control and PCB-contaminated interface. Microbial counts are shown in Table 2-4. Control soil had greater microbial populations than either control or PCB-contaminated interface. The control gravel was examined at UT. A bacterial count of 3.5×10^4 colony forming units was reported (Layton et al. 1994). The microbial populations of gravel and interface are smaller than those of typical agricultural soils (Buckman and Brady 1969). Indigenous bacteria probed with cloned *bph* gene fragments hybridized with 17% and 6-11% of respective PCB-contaminated and control soil (Layton et al. 1994). These findings indicate low potential for natural PCB degradation and a need for bioaugmentation of interface with known PCB-degrading species.

Table 2-3
Characteristics of Bank Number 3 Interface

Parameter	Characterization
Soil texture	26.1% clay 24.7% sand 49.2% silt moderate permeability
pH	8.55
Organic carbon	0.4 wt %
Total carbon	80.109 ppm
Total nitrogen	0.018 wt %
Barium	11.3 ppm
Beryllium	0.17 ppm
Boron	1.88 ppm
Cadmium	below detection
Calcium	25.68 wt %
Chromium	5.54 ppm
Cobalt	1.7 ppm
Copper	14.6 ppm
Iron	5,705 ppm
Lead	22.3 ppm
Magnesium	3,613 ppm
Manganese	72 ppm
Phosphorus	0 ppm
Potassium	22 ppm
Zinc	453 ppm
Vanadium	3.2 ppm
Base Neutrals (GC-MS)	45.3 ppm PCB 1248 (all other Aroclors below detection limits)
Acid Extractables	below detection limit
Volatiles (Methods 8010, 1020)	5.2 ppb Xylenes (all others below detection limit)

Table 2-4
Microbial Plate Counts of Bacteria and Fungi in Interface of Capacitor Bank 3

Sample Description	Bacteria per gram (mean)	Fungi per gram (mean)
Control Soil	86×10^7	1.9×10^6
PCB-Contaminated Interface	4×10^6	7×10^3
Control Interface	2×10^6	4×10^3

Laboratory Tests in Support of a Biotransformation Process

A variety of tests were performed that lead to the conceptualization of an in situ PCB biotransformation process. These included a range from determining potential of indigenous microbes for PCB degradation to testing the effects of material of construction for removing PCBs. In addition, some aspects of technical and economic feasibility of the in situ process were addressed. Evaluations of various types were conducted simultaneously at TVA, UT, and ORNL.

Analytical Methods for Experiments and Pilot Scale Tests

A variety of analytical methods were used at the experimental level and in subsequent pilot scale operation of the in situ soil irrigation process for PCB biotransformation of substation soils. All analyses of solid materials are reported on a dry weight basis. Dry weight was determined by taking tare weights of various containers and drying solids to a constant weight at 100º C.

Microbial Counts

For total microbial counts in soil, interface, and gravel, populations were enumerated using a most probable number method incorporating a plate dilution frequency assay (Harris and Sommers 1968). Bacteria were grown on soil extract agar. Fungi were grown on rose Bengal streptomycin agar.

The protocol for plant counts during pilot scale operations included dilution of samples in phosphate buffer and inoculation of both trypticase soy broth in 1.5% agar (TSBA) plates and plates of TSBA with biphenyl. In the latter case, a biphenyl crystal placed in the middle of the plate provided a "biphenyl atmosphere." These plates were used to enumerate PCB degraders while plates without biphenyl were used for total heterotroph counts. Plates were incubated at 37ºC until colonies appeared. The bacterial colonies were counted, and plates were visually inspected for aberrant colony forms. Counts were performed in duplicate.

Surfactant

Surfactant concentration was determined by a modification of the cobalt thiocyanate active substances method (American Public Health Association 1989).

PCB and Biphenyl

The substation was sampled by removing gravel, interface, or soil with a sterile, stainless steel spoon or a sanitized, stainless steel split spoon corer. Sterile spoons and disposable, sterile pipettes were used to remove specimens during laboratory and pilot scale trials. Liquid or solid samples were stored in amber glass containers with Teflon-lined lids. Analytical samples were either processed immediately or stored at -20º C until the time of analysis. Samples for analysis were subjected to Soxhlet extraction with hexane unless otherwise specified. Hexane extracts were passed over Na_2SO_4 for water removal. Extracts were analyzed on the Varian 3600 gas

chromatograph (GC) equipped with an electron capture detector using the following protocol unless otherwise specified. Separations were accomplished in the splitless mode with a 30 m X 0.25 mm i.d. capillary column of 0.25 µm film thickness (DB-1, J&W, Folsom, CA). The GC conditions were injector temperature of 245º C; detector temperature of 350º C; initial column temperature of 100º C, increased at a rate of 10º C per min until a temperature of 150º C was reached and held for 2.5 min, increased at a rate of 3º C per min until a temperature of 250º C was reached and held for 10 min, increased at a rate of 20º C per min until a temperature of 260º C was reached and held for 2 min. The total run time was 53 min. The carrier gas was He, with N makeup in the detector. Peak identification was based on comparison of unknown peaks to those in standard Aroclor 1248 at concentrations of 0.1, 0.5, and 1 ppm (wt/vol). The identification and weight percent composition for each of 35 chromatographic peaks in Aroclor 1248 was used to interpret changes in PCBs over time (Bedard et al. 1987, Wagner 1994). Congener concentrations were determined on a part per million (ppm) basis, mg of congener or of total congeners per kg of solid or mg of congener or of total congeners per liter of liquid.

Specimens for biphenyl analysis were processed in a similar fashion to those for PCB analysis. Extracts were analyzed using a flame ionization detector and a 0.53 mm DB-5 column in splitless mode with a 30 m X 0.53 mm i.d. column of 1.5 µm film thickness (BD-5, J&W, Folsom, Ca). The GC conditions were the same as those for PCB analysis.

Potential of Indigenous Microbes versus Bioaugmentation for PCB Biotransformation

An ideal in situ PCB biotransformation process takes advantage of the activities of indigenous microbial populations. Experiments were performed to determine whether such populations were adequate or whether bioaugmentation with known PCB degraders is essential for PCB biotransformation of the interface.

Stimulating Indigenous Aerobic Microbial Populations

A trial was performed to determine whether various nutrients that increase microbial populations are effective for improving PCB degradation. A supply of PCB-contaminated interface was supplemented with 200 ppm (wt/wt) phosphorus by dilute phosphoric acid addition. A 40-g sample of the interface was placed in a sufficient number of Erlenmeyer flasks for five replicates of a full factorial test. Variables included incubation of interface with its potential indigenous microbial population as is or sterilizing interface by addition of silver nitrate; the amendments corn meal, soybean meal, or broiler litter at 5% concentration by weight; surfactant, Alfonic 810-60, at 100 ppm (wt/wt) or absence of surfactant; and soil moisture at the approximate water holding capacity of 25% moisture or at saturation. The flasks at 25% moisture were opened and shaken twice weekly for aeration. Flasks were harvested at day 0 and day 100 to evaluate changes in PCBs over time. Interface was extracted with a 9:1 hexane to acetone mix on a magnetic stir plate for 30 minutes. Extracts were concentrated by evaporation and analyzed for PCBs. The chromatographic patterns of PCBs were unchanged over the 100 days incubation in spite of treatment applied. The supplements were unsuccessful for enhancing PCB degradation by indigenous microbes within the 100 days.

A test was performed (Layton et al. 1994) with 1:5 slurries of interface in PAS medium (Bedard et al. 1986) with additions of either biphenyl, 4-chlorobiphenyl (4-CB), and 4, 4'-dichlorobiphenyl (4, 4'-CB); biphenyl, 4-CB, 4, 4'-CB, and known PCB-degrading bacterial species, *Alcaligenes eutrophus* (GG4202); or biphenyl, 4-CB, 4, 4'-CB, GG4202, plus the nonionic surfactant, Igepal CO-720, (Aldrich Chemical, Milwaukee, WI). The slurries were incubated for two-weeks. Bioaugmentation with the PCB degrader *A. eutrophus* GG4202 was necessary for interface PCB degradation. However, when Igepal CO-720 was included with GG4202, there was no PCB degradation. It was concluded from the results that interface PCB biotransformation benefits from bioaugmentation.

These tests further indicate that the interface PCB degrading population is small at best. The addition of PCB degrading microorganisms is needed for a successful in situ process.

Stimulating Indigenous Anaerobic Dechlorination

Klasson et al. (1996) incubated interface samples under anaerobic conditions to determine the ability of the indigenous microbial populations to dechlorinate PCBs. They applied a variety of treatments to enhance dechlorinating activity. Treatment variables included the addition of pyruvate or maleate and use of acetone as a carrier for the addition of the inducers, 2, 3, 6-trichlorobiphenyl and 2, 4, 6-trichlorobiphenyl. At the same time some interface samples were provided an inoculum of dewatered Hudson River sediment and mineral medium. The Hudson River sediment demonstrated PCB dechlorination activity. Data analysis was performed on 68 congener-containing chromatographic peaks and the chlorines per biphenyl were determined as an indication of dechlorination. Incubations were sampled over a 19-week period.

There was no dechlorinating activity in the absence of an inoculum in spite of other treatment variables. This demonstrates the lack of a dechlorinating population in the interface. On the other hand, dechlorination was evident with all treatments that included the Hudson River inoculum. Each carbon source, including the acetone control, supported dechlorination if added with inoculum. However, the chlorobiphenyl inducers actually fostered less dechlorination in combination with carbon sources than the carbon sources alone.

It was concluded from the incubations that an indigenous interface PCB dechlorinating population is small or nonexistent. Bioaugmentation is necessary for PCB dechlorination just as it is for aerobic PCB degradation of interface. The successful transfer of dechlorinating activity to the interface slurries, in the form of Hudson River sediment and mineral medium, has important implications for the in situ PCB biotransformation process.

Bioaugmentation and Biphenyl Addition

The advantages of interface bioaugmentation as well as biphenyl addition were further investigated in a year-long incubation of the interface. Bioaugmentation consisted of either combined PCB-degrading bacteria *Corynebacterium sp.* MB1, *Alcaligenes eutrophus* H850, and *Pseudomonas* sp. LB400; the white rot fungus (WRF), *Phanerochaete chrysosporium*; or these bacteria plus WRF.

Stainless steel pans (22.86 cm wide by 27.94 cm long by 10.16 cm deep) were cleaned with soap and water, rinsed first with acetone then with hexane, and steam sterilized. Two kg of PCB-contaminated interface were placed in each pan, and 100 ml of a 20 ppm biphenyl in acetone solution was mixed into each batch of interface to allow acetone to evaporate and biphenyl to be distributed throughout the interface. Five treatment regimes were followed. These are summarized in Table 2-5.

Table 2-5
Treatment Descriptions in Year Long Pan Incubation

Treatment	Microbes	Nutrient	Supplement
Supplement control	None	None	Wheat straw
Nutrient control	None	Mineral salts medium	None
White rot fungus	*Phanerochaete chrysosporium*	modified Melin Norkrans medium	Wheat straw
Bacteria	*Pseudomonas sp.* (LB400) *Alcaligenes eutrophus* (H850) *Corynebacterium sp.* (MB1)	Mineral salts medium	None
White rot fungus and bacteria	*Pseudomonas sp.* (LB400) *Alcaligenes eutrophus* (H850) *Corynebacterium sp.* (MB1) *Phanerochaete chrysosporium*	Mineral salts medium	Wheat straw

To those pans designated for bacteria, a 50-ml, high density inoculum of each species was added at 0 hour and 6, 14, 24, and 40 weeks. The PCB-degrading bacteria were originally obtained from the American Type Culture Collection, grown to high densities in trypticase soy broth, harvested, and stored at -70º C in 20% glycerol. Pan inocula were prepared by growing large portions of each of the stored bacteria in modified mineral salts medium (MSM), concentrating the bacteria by centrifugation, and bringing the volume of each to 50 ml with fresh MSM. MSM was composed of (g/L) 1.741 K_2HPO_4, 0.414 $NaH_2PO_4 \cdot H_2O$, 1.321 $(NH_4)_2SO_4$, and 0.120 $MgSO_4$ (Hickey and Focht 1990) plus 1 ml of a salts solution at concentrations (g/L) 5.046 $FeSO_4 \cdot 7H_2O$, 0.201 $MnCl_2 \cdot 4H_2O$, 0.107 $ZnSO_4 \cdot 7H_2O$, 0.205 H_3BO_3, 0.010 $Na_2MoO_4 \cdot 2H_2O$, 0.107 $CoCl_2 \cdot 6H_2O$, and 2.054 $CuSO_4 \cdot 5H_2O$. The medium was supplemented with 0.05 g/L yeast extract. At 0 hours, 50 ml of each bacterial concentrate was added to the appropriate pans with stirring. At 6, 14, 24, and 40 weeks, 150 ml MSM supplemented with an additional 2.74 g/L NH_4NO_3 was added to treatments indicated.

A one-time inoculation with WRF was made at 0 hour to the pans indicated. The WRF was originally obtained from Kent Kirk, Forest Products Laboratory, Madison, Wisconsin, and was maintained in continuous passage on potato dextrose or malt extract agar. For pan inocula preparation, malt extract agar plates were inoculated and, subsequently, incubated for seven days. Spores were washed from each plate with a small amount of sterile water, and 1 ml of each spore suspension was added to 200 ml modified Melin Norkrans medium (MMN)

(Viney and Bewley 1990), mixed with 100 g processed wheat straw, and incubated for 7 days. Processed wheat straw was prepared by finely chopping wheat straw and steam sterilizing it. At 0 hour, the inoculated processed wheat straw was added to the appropriate pans and mixed with the interface. The pan indicated as the supplement control received 100 g of the chopped, sterile wheat straw only.

Water was added to the pans at 0 hour and 8, 12, 13, 16, 20, 28, 32, 40, and 44 weeks to keep the interface moist. The pans were covered with aluminum foil at 0 hour, and the coverings were removed only for sampling or making additions to each pan. The 0 hour interface pH among the pans ranged from 7.0 to 8.0. The pH range was 7.03 to 8.8 at 20 weeks as determined by a meter reading of a slurry of two parts water to one part interface.

A solution of 1 g biphenyl in 50 ml acetone was applied to the designated pans using an atomizer at 6, 14, 24, and 44 weeks to maintain the biphenyl concentration about 1000 ppm. The interface was mixed to evaporate the acetone and leave the desired amount of biphenyl.

A modified most-probable-number method was contrived to distinguished bacterial populations that used biphenyl and a mix of congeners as carbon source from populations that use glucose as the sole carbon source. To perform a bacterial count, a specified amount of either biphenyl and congeners dissolved in acetone or glucose in water was placed in triplicate test tubes along with chlorine-free medium (Adams et al. 1992). Tubes thus contained either the biphenyl and congener mix (1250 ppm biphenyl; 21.2 ppm 2, 3-dichlorobiphenyl; 0.3 ppm 2, 5, 4'-trichlorobiphenyl; 42.0 ppm 2, 3, 2' 3'-, 8.7 ppm 2, 5, 3', 4'-, and 0.3 ppm 2, 5, 2', 5'-tetrachlorobiphenyl; and 0.3 ppm 2, 4, 5, 2', 5'-pentachlorobiphenyl) or 1000 ppm glucose (wt/wt). The test tubes were inoculated with dilutions of interface in buffer at various times over the course of the incubation. The glucose-utilizing population was viewed as a reflection of the total bacterial population. The biphenyl and congener-utilizing population served as a reflection of the PCB degrading population. At the same time, dilutions of the interface were plated on rose Bengal agar to estimate the fungal, assumed to be WRF, population.

The pans were sampled for PCBs, biphenyl, bacteria, and fungi at a total of 20 time intervals over the course of incubation. Decisions to reapply bacterial inocula and biphenyl at the time intervals previously indicated were based on the analytical results. A careful record was kept of the weights of samples withdrawn. At the end of incubation, the remaining interface in each pan was weighed and analyzed for PCBs. The pans were given a final hexane wash, and surfaces were scraped with rubber policemen. The scrapings were weighed and analyzed for PCBs. Data collection allowed the time course of changes in microbial populations, biphenyl, pH, and PCB concentration to be observed and a mass balance for PCBs to be determined.

Table 2-6 contains the log values of total and degrader bacterial populations over the course of the incubation. The total bacterial population was similar in all treatments over the course of the incubation. However, the total population was somewhat smaller initially in the supplement control than the other treatments. The degraders constituted a large portion of the total bacterial population, in the early part of the incubation, in the treatments receiving the PCB-degrading bacteria. Over time, the degrader population decreased, relative to the total population, in all treatments except the supplement control. The degrader population increased in numbers relative to the total population in the supplement control.

Table 2-6
Changes in the Total and PCB-Degrading Bacterial Populations Over Time during the Year-Long Pan Incubation

Treatment	Sample time Interval (weeks)	Log total Bacteria	Log degrading Bacteria	Degraders (% of total Population)
Supplement	6	5	1	20
Control	8	6	1	17
	10	8	1	13
	12	8.4	1	12
	14	7.4	1.4	18
	16	8.0	5	63
	18	8.2	6	73
	20	8.4	7	84
	24	11	9	82
	28	11.7	6.6	57
	32	8.4	6.4	76
	36	7.4	7.4	100
	40	8.2	7.4	90
	44	7.0	7.4	106
	48	9.4	4.4	46
	52	10.4	5.4	52
Nutrient	6	8	7	88
Control	8	8.7	6.2	71
	10	8.4	5	60
	12	8.5	2	24
	14	7.3	2	27
	16	6.9	2.4	34
	18	9	5	56
	20	8.6	6.0	70
	24	11	4	36
	28	10.5	3.4	32
	32	8.4	2.4	28
	36	8.0	1.4	17
	40	10.4	1.4	13
	44	9.0	2.4	26
	48	10.4	1.4	13
	52	10.4	1.4	13
White rot fungus	6	9	4.6	51
	8	10	6.4	64
	10	11	6.4	58
	12	9.4	6.4	68
	14	8.3	6.4	77
	16	9.6	7	73
	18	8.6	5.4	62
	20	7.8	5.6	72
	24	11	5.4	49
	28	9.7	3.4	35
	32	8.4	2.4	28
	36	8.6	2.6	30
	40	9.7	5.4	56
	44	9.0	2.4	26
	48	10.4	2.4	23
	52	10.4	1.4	13
Bacteria	6	8	8	100
	8	9.4	9.4	100
	10	8.0	6.9	86
	12	10.0	5.6	55
	14	7	3	43

Table 2-6
Changes in the Total and PCB-Degrading Bacterial Populations Over Time during the Year-Long Pan Incubation (Continued)

	16	8.7	6.0	70
	18	8.9	5	56
	20	8.0	4.4	55
	24	8.0	4.6	58
	28	10.7	6.4	60
	32	7.4	4.4	59
	36	8.4	1.4	16
	40	10.4	2.4	23
	44	7.4	2.4	32
	48	10.4	2.4	23
	52	10.4	1.4	13
Fungus and	6	8	8	100
Bacteria	8	10.0	8.6	87
	10	8.4	8.4	100
	12	10.8	8.4	78
	14	8.2	6	73
	16	9.6	7	73
	18	8.6	5.2	60
	20	8.6	5.0	57
	24	8.9	4.4	49
	28	6.6	6.0	92
	32	5.4	3.4	63
	36	7.4	5.4	73
	40	9.4	4.6	49
	44	6.4	2.4	37
	48	10.4	2.4	23
	52	10.4	1.4	13

Table 2-7 summarizes the major trends that the fungal population followed in each of the treatments. Again, it is of interest to note that over the incubation both a PCB-degrading bacterial population and a fungal population arose in the supplement control. The appearance of the latter populations was not anticipated. The supplement control was not supplied with sources of nitrogen or phosphorus. The supplement control was intended to be a means to judge whether PCB disappearance were due to degradation by WRF as opposed to sorption to the supplemental processed wheat straw. The time course of the supplement control incubation shows that the degrader bacterial population reached a peak concentration at 24 weeks and the fungal population reached a peak at 12 weeks (data not shown). It cannot be ruled out that the supplement control decline in PCBs from 24 through 52 weeks was due to bacterial activity. Therefore, the supplement control did not serve as a sterile or killed control as was intended.

Table 2-7
Survival of Fungi under various treatments in the Year-Long Incubation

Treatment	Log of Fungi per ml			
	2 weeks	14 weeks	24 weeks	52 weeks
Supplement control	0	4.9	4	2.5
Nutrient control	0	3.2	3.2	0
White rot fungus	5.6	5.2	6.6	5.5
Bacteria	0	0	1.3	0
White rot fungus and bacteria	5.7	6.7	6.9	4.3

The pans were sampled 19 times during the year-long incubation and a twentieth and final sampling was made of the bulk residual interface in each pan and the scrapings of the pan walls. The PCB congeners represented by each of the 35 chromatographic peaks are identified in Table 2-8. The 19 values for each of 35 peaks were subjected to the student t test of the hypothesis that the steady decline in the congener loss over the year reflected only normal variation around the 0 hour analysis. Thirty-five peaks representing 71 congeners were evaluated. The weight percentages that individual peaks comprise of the total PCB in the contaminated interface are shown. All the chromatographic peaks found in detectable amounts in the contaminated interface decreased in concentration over the time course. Peaks 1, 2, 4, and 5 were not detected. The hypothesis that this disappearance may be due to sampling variability was tested. The student t test was applied. If variability were not outside the 95% confidence interval, the degradation percentage is shown as zero in Table 2-8 in spite of the fact that all peaks decreased in concentration over the incubation.

Table 2-8
Percent Congener Degradation Over the Year-Long Incubation (Although the Amount of All Congeners Decreased Over the Incubation, only Statistically Significant Decreases were considered due to Degradation)

		TREATMENTS					
			Supplement Control	Nutrient Control	White rot fungus	Bacteria	White rot fungus and bacteria
Peak	Congener(s)	Weight percent of total PCB	Statistically significant degradation over the year-long incubation (%)				
1	2,5,2'; 4,4'	0.0	NA	NA	NA	NA	NA
2	2,4,2'	0.0	NA	NA	NA	NA	NA
3	2,3,2'; 2,6,4'	0.2	55	0	0	57	70
4	2,5,3'	0.0	NA	NA	NA	NA	NA
5	2,5,4'	0.0	NA	NA	NA	NA	NA
6	2,4,4'; 2,4,2',6'	2.8	0	76	83	83	81
7	2,3,3'; 2,3,4; 3,4,2'; 2,5,2',6'	0.1	0	66	0	0	69
8	2,3,4'; 2,4,2',6'	0.8	0	64	75	74	82
9	2,3,6,2'	0.4	49	0	0	57	69
10	2,3,2',6'	0.3	69	0	0	0	100
11	2,5,2',5'; 2,3',5',6'	2.3	46	0	0	55	73
12	2,4,2',5'	2.5	38	0	0	70	74
13	2,4,2',4'	1.5	0	0	0	47	0
14	2,4,5,2'; 2,4,6,4'	0.9	0	100	0	72	0
15	2,3,2',5'; 2,4,6,2',6'	3.1	0	61	0	83	78
16	3,4,4'; 2,3,2',4'; 2.3,6,3'	2.5	0	63	88	84	80
17	2,3,4,2'; 2,3,6,4'; 2,6,3',4'; 2,5,3',5'	5.3	34	0	0	45	70
18	2,3,2',3'	1.2	0	74	89	87	83
19	2,3,3',5'	1.1	0	62	83	84	86
20	2,4,5,4'	6.1	0	40	0	79	80
21	2,5,3',4'; 3,4,5,2'; 2,3,4,5'	2.5	0	0	0	68	67
22	2,4,3',4'; 2,3,5,6,2'; 2,4,6,4'	16.8	0	63	87	85	77
23	2,3,6,2',4'; 2,4,2'3'6'; 2,3,3'4'	1.1	0	0	0	60	86
24	2,3,3',4'; 2,3,4,4'	11.4	0	61	0	84	83
25	2,3,6,2',3'; 2,3,5,2',5'; 2,4,6,2'4'6'	1.2	0	0	0	57	72
26	2,3,5,2',4'; 2,4,5,2',5'	3.0	0	0	0	60	74
27	2,4,5,2',4'	2.7	0	0	0	40	0
28	2,4,5,2',3'; 2,3,5,6,2',6'; 2,4,5,2'3'	1.9	0	67	88	88	87

Table 2–8
Percent Congener Degradation Over the Year-Long Incubation (Although the Amount of All Congeners Decreased Over the Incubation, only Statistically Significant Decreases were considered due to Degradation) (Continued)

29	2,3,4,2',5';2,3,5,3',5'; 2,3,4,6,4'	2.6	0	0	0	43	0
30	2,3,4,2'4'; 2,3,4,5,6	2.5	0	0	0	0	0
31	2,3,6,3'4'; 3,4,3',4'	5.7	0	0	0	0	0
32	2,3,4,2',3'	1.9	0	61	0	87	85
33	2,3,6,2',4',5'; 2,4,5,3',4'; 2,3,4,5,3'	7.0	0	0	0	71	81
34	2,3,4,3',4'; 2,3,4,2',3',6'	6.1	0	0	0	65	87
35	2,3,4,2',4',5'; 2,3,5,6,3',4'; 2,3,4,5,6,3'	2.5	0	0	0	0	0

Treatments consisting of the addition of bacteria and bacteria plus WRF contain the most peaks with statistically significant loss over the time period. The congener composition at 0 hour shows some peaks in high abundance relative to others. It did not follow, however, that peaks of relatively high concentration are the ones showing the greatest loss in concentration over the incubation period. No pattern suggesting that peak disappearance is a matter of dilution is indicated.

The PCB mass balance is presented in Table 2-9. A similar statistical analysis of the total of all chromatographic peaks at each sampling was performed. The hypothesis that the losses of total PCBs experienced over the incubation were simply variations around the true value (the 0 hour value) was tested. The student t test was applied. It is concluded that the 32, 25, and 41% degradation by respective supplement control, nutrient control, and fungal addition may be statistically interpreted as due to normal variation around the 0 hour total PCB concentration. However, the two treatments which include the PCB-degrading bacteria are interpreted as statistically significant losses of PCBs. The treatments consisting of addition of bacteria and of bacteria and WRF each resulted in 57% PCB degradation.

Table 2-9
PCB Mass Balance of Year-Long Incubation of Interface

	Treatment				
	Supplement Control	**Nutrient Control**	**White rot Fungus**	**Bacteria**	**White rot Fungus and Bacteria**
PCB at outset of incubation (mg)	100.52	56.89	196.66	130.84	91.86
PCB accounted for in 20 samples (mg)	42.95	30.21	83.65	47.80	31.78
PCB at end of incubation including hexane wash of pans (mg)	25.53	12.40	32.99	8.00	7.68
PCB unaccounted for and assumed degraded (mg)	32.04	14.28	80.02	75.04	52.40
Degradation (%) (PCB unaccounted for/initial PCB)*100	32	25	41	57	57

The rates of PCB loss for each treatment were determined (data not shown). The treatments with added bacteria alone or bacteria in combination with WRF showed a significant decline in PCB concentrations between 0 hour and 2 weeks. All treatments showed a noticeable decline in PCB concentrations beginning around 28 or 32 weeks. A great deal of the PCBs were accounted for in the residual interface at the end of incubation in all treatments.

The pan test results further confirm the necessity of bioaugmentation of the PCB-contaminated interface. The results indicate that bacteria, as opposed to WRF, were more effective for promoting PCB degradation. The necessity of the presence of biphenyl for aerobic degradation was demonstrated. The test results showed the potential of an in situ process incorporating aeration and repeated addition of nutrients and bacterial inocula. However, the extended incubation time required for PCB degradation has negative implications for an in situ process.

A second pan test was performed with a similar design and these exceptions. The single control pan contained PCB-contaminated interface with no addition of biphenyl, wheat straw, or nutrient. There was no test of combined PCB-degrading bacteria plus WRF. The surfactant that was later used in the process, polyoxyethylene 10-lauryl ether, referred to as POL (Ethal LA-12, Ethox Chemicals, Inc., Greenville, SC), was added to the interface at a concentration of 600 ppm. The interface was incubated for 32 weeks.

At the end of 32 weeks, there was an increase in the PCB concentration of the control that was given no additives to stimulate or augment PCB degradation. There was a decrease in the PCB concentrations of treatments with PCB-degrading bacteria and with WRF. However, the data showed much variability over the time course. There was no statistically significant loss of PCBs in any treatment as determined by a test of the hypothesis that loss reflects variability around the 0 hour value for PCBs. These results contrast with the first pan test where the presence of PCB-degrading bacteria brought about a statistically significant loss of PCBs by 32 weeks of incubation. At least two trends may be inferred from the second test. The control in the second test shows that PCBs remain unchanged in unamended interface. This suggests that the PCBs loss from the supplement control pan in the first test is likely due to the appearance of degrader populations. It is concluded that bioaugmentation stimulates PCB loss in the interface and such loss is due to biological factors. The lack of statistically significant PCB loss with the PCB-degrading bacteria in the second pan test suggests that surfactant may have inhibited the bacteria in the second test as opposed to the bacteria in the first test which received no surfactant. The effects of surfactants are discussed elsewhere in this report. These conclusions from the pan tests further support the need for bioaugmentation and, ultimately, the of the recombinant bacteria, the field application vectors (FAVs), in the in situ soil irrigation process for PCB biotransformation in soils.

FAVs

To preclude the necessity of repeated biphenyl addition (Brunner et al. 1985), the use of field application vectors (FAVs) was explored (Lajoie et al. 1993). The strategy was investigated as a means to provide bioaugmentation capacity, shorten the incubation time, save the costs of prolonged operations, avoid the need for repeated biphenyl addition, and overcome problems of bioavailability.

FAVs are recombinant bacterial strains that express non-adaptive genes in a competitive environment. FAVs are favored in particular environments because the bacteria thrive on a substrate that actually selects for their survival. For application to interface PCB degradation, FAVs were produced as surfactant-degrading bacterial strains containing either plasmid- or transposon-based PCB-degradative genes. A constitutive promoter was selected to uncouple the need for the inducer, biphenyl, from PCB degradation.

For the application described herein, several traits were sought for the FAVs. The selective substrate is a surfactant for soil washing to recover PCBs, a linear alcohol ethoxylate surfactant that is mid-range in ethoxylate and alkyl chain length. Surfactants of this composition are considered moderately degradable at environmental levels of 1-100 ppm (Larson and Games 1981). The FAVs developed survive at much higher surfactant concentrations. The in situ process for biotransformation of PCBs developed makes use of 10,000 ppm (wt/vol) surfactant solution for interface washing. Abdul and Gibson (1991) demonstrated that 10,000 ppm is an optimum concentration for soil washing. This concentration makes the surfactant solution a selective medium for the FAVs, discouraging the growth of other microbes. The host strain from which the FAVs were produced degrades susceptible PCB congeners (Lajoie et al. 1994).

Developing the Recombinant Strains

The parent organisms for development to FAVs were characterized as *Pseudomonas putida* IPL5 and *Ralstonia eutrophus* B30P4. Details of the characterization is contained in correspondence with EPA Headquarters and is archived in Muscle Shoals. A chromosomally-based transposon was inserted to effect PCB degradation. The recombinants have been described in presentations by EPA as well (Sayler and Sayre 1995).

Surfactant

The FAVs were ultimately developed around the use of one particular surfactant, polyoxyethylene lauryl ether or POL. Investigations of properties of a number of surfactants were performed to arrive at the use of POL in PCB biotransformation.

Surfactant-Aided PCB Recovery

Surfactants were tested for benefits in PCB recovery from interface. A100-ml volume of a 10,000 ppm solution (wt/vol) of each of 12 surfactants was mixed with 20-g of interface. The surfactant-interface slurries were placed in 250-ml Erlenmeyer flasks that were covered with foil and Teflon caps. Surfactants included polyoxyethylene alcohols (Brij 30 and Brij 35), polyoxyethylene alkylphenols (Triton X-100, Hyonic PE-90, Tergitol NP-10, and Igepal CO-720), polyoxyethylene esters of fatty acids (Tween 20 and Tween 80), a blend of polyoxyalkylated fatty acid esters (Adsee 799), sodium dodecyl sulfate (SDS), and Simple Green (Sunshine Makers, Inc., Huntington Harbor, CA). The surfactant to soil ratio tested was chosen because Viney and Bewley (1990) reported that they found no additional desorption of PCB from freshly contaminated sand at higher surfactant concentrations.

The flasks were shaken in a rotary shaker, 10 rpm, at room temperature for 24 hours and then held stationary for 24 hours at room temperature. At this point, the surfactant layer was decanted. Fresh surfactant was added, and the flasks were shaken for 96 hours and held stationary for the following 24 hours. Again, the surfactant was decanted, fresh surfactant was added, and the flasks were shaken for an additional seven days and held stationary for the following 24 hours. Each fraction of recovered surfactant was further extracted with hexane through three repeated applications of sonication in an ice bath to break the emulsion. The hexane extracts were analyzed for PCB.

PCB recovery efficiencies were calculated on the basis of PCB in surfactant solution compared to PCB in the unprocessed interface. The recovery efficiencies are shown in Table 2-10. The recovery efficiency with Simple Green and water was small. The best recovery efficiency was achieved with the nonionic surfactant Triton X-100, a polyoxyethylene lauryl ether.

Table 2-10
Efficiency of PCB Recovery from Contaminated Interface with various Surfactants

Surfactant	PCB Recovered (%)
Triton X-100	81.4
Brij 30	75.2
Brij 35	83.5
Hyonic PE-90	66.9
SDS	45.3
Tween 80	79.5
Tween 20	55.4
Tergitol NP-10	68.2
Igepal CA-720	62.5
Igepal CO-720	66.1
Adsee 799	31.3
Simple Green	11.9

The selection of the 10,000 ppm surfactant dose for interface PCB recovery was verified in this fashion. The CMC of various surfactants was determined in both aqueous solution alone and in aqueous solution mixed 1:1 with interface. Both type solutions were sampled and further diluted in distilled water. The surface tension of the dilutions was measured with a tensiometer (Fisher Scientific, Pittsburgh, PA). The apparent surface tension reading (dynes/cm) was plotted versus the log of the corresponding surfactant dilution (data not shown). The break point of each

curve is reported as the CMC value (Table 2-11). The CMC values in aqueous solution for all the surfactants are well below 10,000 ppm, indicating that micelles will form at 10,000 ppm. However, in 1:1 mixes of surfactant solutions and interface, the apparent CMC values were greater than for aqueous solutions. Surfactants adsorb onto soil particles and thus decrease the available surfactant molecules for micelle formation. In the cases of Tween 20 and Tween 80, maximum solubilization of PCB is not achieved at 10,000 ppm surfactant. For all other surfactants tested, 10,000 ppm should be a satisfactory concentration for PCB recovery from contaminated interface.

Table 2-11
Experimentally-Determined CMC Values in Aqueous Solution and in a 1:1 mix of Aqueous Surfactant Solution and Interface

Surfactant	Experimentally-determined CMC (ppm)	Experimentally-determined CMC as a consequence of mixing equal amounts of Aqueous Aurfactant and Interface (ppm)
Triton X-100	100	400
Brij 30	25	1000
Brij 35	80	1050
Hyonic PE-90	100	800
Tween 80	180	>10,000
Tween 20	100	>10,000
Tergitol NP-10	40	900
Igepal CA-720	200	410
Igepal CO-720	70	600
POL	20	1000

More extensive PCB recovery tests were performed with POL. The objective of the further tests were to use a relatively small surfactant wash volume in comparison to the amount of PCB-contaminated interface. Such a strategy can be to keep costs down at increased scales of operation. Several batch surfactant wash schemes to recover PCBs were tested. A laboratory apparatus was designed allowing POL solution to be added to interface, held on the interface for a designated time, and drained for liquid recovery. Two 2000-ml Pyrex beakers were outfitted with a port at the lowest possible point on the side of the beaker. The port consisted of a six-inch glass tube with a Teflon stopcock. A 25 mesh stainless steel screen was inserted into the tube to prevent fines reaching the stopcock. The beakers were placed on a stand and tilted so that the port was perpendicular to the bench top. Contaminated interface (1075 g) with a concentration of

329 ppm PCB (wt/wt) was placed in each beaker. The water holding capacity or pore volume was determined (Klute 1986) using a suction cell apparatus employing a ceramic plate (Soil Moisture Equipment Corporation, Santa Barbara, CA). The water holding capacity of the interface was found to be 100 g/kg.

The effects of interface-POL contact time on PCB recovery were evaluated. Two wash schemes were tried. For the 10,000 ppm POL wash scheme, the stopcock was closed and 7.2 pore volumes of 10,000 ppm POL solution were added to the interface. The POL solution was held on the interface for 4 hours. The interface was then drained, and the POL solution was returned to and held on the interface for a second 4-hour interval. The process was repeated for an additional 16-hour hold time followed by a final 4-hour hold time. The 7.2 pore volumes were recovered. The interface was given a final wash with 2.4 pore volumes of fresh 10,000 ppm POL which was held on the interface for 4 hours. The 20,000 ppm POL wash scheme was implemented in a similar manner except that initially 3.6 pore volumes of 20,000 ppm POL were added. The final POL wash was six pore volumes of a 4000 ppm POL solution. The PCB content of the POL wash was determined after each pass through the soil.

For the 10,000 ppm POL wash scheme, about 25% of the PCB was recovered after washing with the equivalent of 31.2 pore volumes, mainly comprised of recycled POL solution. A linear regression curve fit of the data (figure not shown) predicts complete PCB recovery with 140 pore volumes. About 43% of the PCB was recovered with the equivalent of 20.4 pore volumes for the 20,000 ppm POL wash scheme. A linear regression curve fit of the second wash (data not shown) predicts complete PCB recovery with 53 pore volumes. For both the 10,000 and 20,000 ppm POL wash schemes, the greatest loss of PCBs took place with the first application of POL solution, held on the interface for 4 hours. The three subsequent recycles of the same POL solution with respective holding periods of 4, 16, and 4 hours did not yield as great a recovery of PCBs as the first application of POL solution. However, the final wash with fresh POL solution with a 4-hour holding time provided additional PCB recovery at a rate similar to the first POL application. The extended contact time of 16 as opposed to 4 hours was not beneficial for PCB recovery. The data extrapolation indicates that PCB recovery can be achieved without renewing the POL wash solution, thus minimizing total liquid volume. Although 20,000 ppm POL provided better PCB recovery in this experimental design than 10,000 ppm POL, the potential toxicity of 20,000 ppm POL to a biological process was considered. The 10,000 ppm POL concentration was selected for the in situ biotransformation process.

An additional laboratory scale wash test recycled 10 pore volumes of a 10,000 POL solution repeatedly through interface for a two-day period. POL solution was pumped onto the interface at a rate of 60 ml/min for 30 minutes followed by a 30 minute period for solution to drain from the interface. Approximately one pore volume of liquid remained with the interface and was not recovered. The PCB recovery efficiency was 74% (Beck et al. 1996).

Effects of Surfactants on Microorganisms

The effects of surfactants on the growth of several microorganisms was determined. The WRF and a fungal isolate from the interface, tentatively identified as a *Fusarium* sp., were maintained on potato dextrose agar plates. The *Fusarium* sp. isolate produced a clear zone on a nutrient agar

plate that had been sprayed with biphenyl indicating degradation of biphenyl. Malt extract broth was inoculated with WRF and with *Fusarium* sp.; the biomass was recovered after four days; the biomass was chopped in a Waring blender; and the biomass was used to inoculate either malt extract broth containing 10,000 ppm of each of 11 surfactants or malt extract broth alone. Surfactants included Triton X-100, Brij 30, Brij 35, Hyonic PE-90, Tween 80, Tween 20, Tergitol NP-10, Igepal CO-720, Adsee 799, SDS, and Simple Green.

Biomass grown with and without the surfactants was harvested, dried, and weighed. *Fusarium* sp. biomass accumulation in response to each of the surfactants was either comparable to or exceeded that of the control without surfactant except in the cases of Brij 30 and SDS (data not shown). However, in the case of the WRF, only Tween 80 produced WRF biomass in an equivalent amount to the broth alone. All the other surfactants apparently inhibited growth of WRF. Viney and Bewley (1990) found some surfactants efficient at removal of PCBs but of no advantage for enhancing PCB degradation by a mixed culture of *Ph. chrysosporium* and selected bacterial strains in sand with freshly applied Aroclor 1242.

The WRF was selected for the year-long incubation reported herein because of its published acclaim as a PCB degrader. The PCB degrading ability of *Fusarium* sp. was never confirmed in spite of its growth on biphenyl. *Fusarium* sp. was not used for further testing.

Lajoie et al. (1997) tested the ability of Brij 30, POL, and Igepal CO-720 at 2000 ppm to support growth of *P. putida* IPL5 and *R. eutrophus* B30P4. *P. putida* IPL5 and *R. eutrophus* B30P4 used Brij 30 and POL as growth substrates. *P. putida* IPL5, but not *R. eutrophus* B30P4, used Igepal CO-720 as a growth substrate. Various degradation products were identified by GC/MS analysis, confirming the degradation of the surfactants. Layton (1995) grew combined FAVs *P. putida* IPL5::TnPCB and *R. eutrophus* B30P4::TnPCB in two-liter batches of PAS medium with either 4000, 5000, or 10,000 ppm POL with resulting respective POL degradation rates of 87, 96, and 78%. The rates reflect results at the end of a 96-hour incubation for 4000 and 5000 ppm POL, but 54 hours for 10,000. In the latter case, the incubation was stopped at 54 hours because of foaming. Growth was reflected by an increase in cell density at OD 600 from a reading of 0.1 to respective readings of 2.2, 1.8, and 2.4 for 4000, 5000, 10,000 ppm POL.

Reeves (1997) and Reeves et al. (1995) evaluated the effects of POL on anaerobic dechlorination of Aroclor 1242. They added various inocula with dechlorinating activity to small reactor vessels. The vessels were provided diatomaceous earth and either 200 and 1000 ppm POL (wt/vol). Some vessels were agitated and others held stationary for over 52 weeks. They found that a stationary incubation without POL reduced 200 ppm Aroclor 1242 to less than 10 ppm. Aroclor 1242 was reduced from 200 to 100 ppm with the addition of both 200 and 1000 ppm POL whether incubations were stationary or stirred. POL had an apparent inhibitory effect on anaerobic dechlorination of PCB.

In summary, the various surfactants did not improve or may even have inhibited the growth of the microorganisms investigated and inhibited their anaerobic dechlorination of PCB. However, the FAVs, *P. putida* IPL5::TnPCB and *R. eutrophus* B30P4::TnPCB, showed a good growth response to POL. More importantly, *P. putida* IPL5::TnPCB and *R. eutrophus* B30P4::TnPCB degrade PCBs in the presence of the surfactants, as discussed in subsequent sections of this report.

Diatomaceous Earth

There are no reports in the scientific literature of sustained anaerobic PCB dechlorination in the absence of soil or sediments. Wu and Wiegel (1997) described the presence of soil as a requirement for successful transfer of dechlorinating activity to fresh soil. Cutter et al. (1998) were able to demonstrate *ortho* dechlorination with sediment-free inocula. This was accomplished by holding the sediment-free cultures for two to five months between transfers to fresh sediment. However, they determined that better dechlorination results were reached with the addition of sediment. Likewise, Holoman et al. (1998) found that deletion of sediment from PCB-dechlorinating enrichment cultures reduced the rate of dechlorination and diversity of the community. The exact role of the soil and sediment is not understood. For example, Cutter et al. (1998) tried supplementing two commercial humic acids for sediment and found the humic acids and also anthraquinone-2,6-disulfonic acid, an intermediate electron acceptor in the facilitation of biological Fe^{3+} reduction, completely inhibited dechlorination. Whatever the benefits of sediment may be, they are thought to be more complex than as a surface for contact. However, providing a surface for contact with the yet-to-be-identified anaerobic species or consortia responsible for PCB dechlorination may be a role of sediment. Diatomaceous earth was selected as a replacement for soil or sediment for the introduction of dechlorinating activity in the laboratory and pilot scale tests involved with development of the in situ soil irrigation process for PCB biotransformation. The diatomaceous earth was to provide a surface for the dechlorinating activity from Hudson River supernatant liquid.

In addition, diatomaceous earth was incorporated in aerobic degradation of the in situ soil irrigation process as a means to sequester PCB from liquid and move them to the anaerobic dechlorination cycle. This sequestering of PCBs on solids allows a reduction of the total liquid volume of the process. The value of diatomaceous earth for sequestering PCBs was tested. Stainless steel beakers were fabricated to simulate the pilot scale bioreactor material of construction. The amount of PCB congeners in liquid and solid phases and on the stainless steel surfaces following a bacterial degradation cycle and a liquid-solids separation were determined. The starting material was an interface wash solution with 135 ppm PCBs in 10,000 ppm POL solution.

The wash solution was placed in the stainless steel beakers and incubated under conditions similar to those proposed for large-scale operation. Test variables included 5 g/L diatomaceous earth; nutrient solution composed of PAS medium and PA salts (Bedard et al. 1986); and FAVs, *P. putida* IPL5::TnPCB and *R. eutrophus* B30P4::TnPCB. Five treatments were applied in the beakers (Table 2-12). The beakers were agitated on an incubator-shaker. Samples were taken periodically during the incubation. At 130 hours, the contents of the beakers were harvested for separation of liquid and solids by centrifugation. Teflon policemen were used to scrape the beaker walls. The beakers were rinsed with hexane prior to GC analysis of the various solids and liquids harvested. Weights and volumes of samples and residual liquids and solids were recorded for use in calculating the material balances.

PCBs remained with solids exiting the separation process when an inoculum of FAVs was included in the treatment. Less PCB remained on the walls of the reactor when diatomaceous earth was included with FAVs than with FAVs alone. However, if the FAVs were not included in the process, PCBs remained in solution after the separation stage.

Table 2-12
Effects of Biodegradation and Separation Processing Procedures on Fate of PCBs

Portion of the original PCB found in Final Fractions	Treatment additives				
	FAVs, Diatomaceous Earth, PAS and PA	FAVs, PAS and PA	Diatomaceous Earth, PAS and PA	PAS plus PA	None
PCB in final liquid (%)	15	10	74	84	90
PCB on solids (%)	72	51	6	1.5	1
PCB on reactor walls (%)	1	5	2	1.5	1
PCB unaccounted/biodegraded (%)	12	34	18	13	8

In treatments with FAVs present, with and without diatomaceous earth, resulted in respective recoveries of 72 and 51% of PCBs with solids. In these cases, the exit POL concentration of 6700 ppm is much above the CMC of 1000 ppm. However, the PCBs were not associated with the liquid fraction in spite of this POL concentration. The results suggest that the PCBs may be associated with biomass rather than the diatomaceous earth. A plausible explanation is that centrifugation broke the emulsion causing the PCBs to be released from the micelles. However, because centrifugation removed the biomass along with the diatomaceous earth in these supplementary tests, it cannot be ruled out that the PCBs were not associated with the biomass.

POL concentrations were virtually unchanged over the time course in all treatments without the FAVs. Without the FAVs, from 1 to 6 of PCBs reside with solids. Most of the PCBs remain with the liquid as predicted. With FAVs, 72 and 51% of the PCBs, in respective treatments with and without diatomaceous earth, are found with solids. The results support the fact that metabolism of POL must be optimum in order that no PCBs exit in the liquid.

The materials balance was based on the concentration of PCBs in the wash solution originally placed in each beaker. It was found that there were PCBs unaccounted for after processing in the case of every treatment. This loss of PCBs was attributed to biodegradation. The portion of PCBs degraded ranged from 8 to 34%. The 34% degradation complements laboratory results of 35% degradation previously reported (Beck et al. 1996).

The treatment consisting of FAVs and diatomaceous earth was expected to degrade PCBs to the same extent as the treatment with FAVs without diatomaceous earth because each of the treatments metabolized the same relative amount of POL (data not shown). However, this was not the case. The treatment consisting of FAVs with diatomaceous earth did not degrade as much PCB as the treatment without diatomaceous earth. Possible explanations are that the 130-hour incubation was less than optimum, the bacteria did not reach maximum activity, and the culture of FAVs had resulted in lost PCB-degrading capacity. To resolve the issue, a second and similar test was performed. In the second test, the bacteria removed the same amount of PCB from the liquid phase during a protracted incubation period of 480 hours both with and without diatomaceous earth.

In the first experiment, 5% of the input PCB adhered to the vessel wall with the addition of FAVs without diatomaceous earth. This 5% portion of PCBs attached to the wall is not statistically significant compared to 1 to 2% in the other cases. However, it is believed that the 5% of PCBs found on the wall is due to attachment of biomass which has adsorbed PCB. The

test results indicate that it is possible to make reasonable material balances. There should be no concern that PCBs will be lost on the equipment and erroneously interpreted as lost due to biological activity.

The results of support the conclusion that diatomaceous earth may not be necessary as a means to carry the residual PCBs forward after the separation step. Biomass may serve to capture the PCBs and carry them forward. However, diatomaceous earth was used in the pilot scale test reported herein.

Layton et al. (1998) reported that with genetically engineered strains without genes for PCB degradation, *P. putida* IPL5::TnTc and *R. eutrophus* B30P4::TnTc, 84% of PCB is associated with the solids at the end of an incubation with 10,000 ppm POL.

Centrifugation was originally planned for the liquid-solids separation at pilot scale. During shakedown runs, attempts were made to recover diatomaceous earth and biomass using a DeLaval Gyro-Tester bench top, high speed laboratory centrifuge (Alfa Laval, Lund Sweden). The centrifuge efficiently removed diatomaceous earth but not bacteria. Diatomaceous earth was removed so well that it adhered to the internal parts of the centrifuge. The centrifuge had to be frequently dismantled to manually scrape diatomaceous earth off the parts. It was decided that centrifugation not be used in the pilot scale demonstration because of potential for introducing error in the materials balance and problems with decontaminating and restoring the centrifugation equipment to use after exposure to PCBs. Additionally, diatomaceous earth caused pumping problems with the tubing due to its abrasive nature.

Approval for Inclusion of Recombinant Microorganisms in Research and Development

The goal of the project was the eventual field demonstration of an in situ process for biotransformation of PCBs in soils. Ongoing discussions were conducted with the EPA in order to secure approval for such a field demonstration. The inclusion of recombinant microorganisms for eventual field deployment was subject to approval by EPA Headquarters. EPA considered the level of information needed for approval of research and development involving recombinant microorganisms equal to a well-designed scientific experiment. The correspondence with EPA is archived in Muscle Shoals. The responses to EPA address such issues as quality assurance, chain of custody, history of the FAV production, methods of congener-specific PCB analysis, and methods for containment of the bacteria. According to EPA, this project comprised the first request for approval of recombinant microorganisms for bioremediation in the field. The response to inquiries by EPA about deployment of a field test took place from about December 1993 through August 1996. A rather large amount of detailed information was delivered to EPA because there were no standard validation strategies for commercialization of a PCB bioremediation process. EPA intended to evaluate requests for use of recombinant microorganisms case by case. For potential commercialization of the process, EPA considered PCB destruction to be the equivalent of reduction in the PCB concentration to less than 2 ppm per congener peak quantified. Because destruction to this level was not achieved in laboratory and pilot scale tests, the pursuit of the approval for a field test was abandoned. EPA staff used the project as an example of the type of information that is needed for similar field demonstrations (Sayler and Sayre 1995).

3
PILOT SCALE TEST OF AN IN SITU PROCESS FOR BIOTRANSFORMATION OF PCBs IN SOILS

The results of laboratory tests and ongoing dialogue with EPA led to the formulation of an in situ soil irrigation process for the biotransformation of PCBs in soils. The process features several unit operations. The process includes surfactant soil washing to recover PCBs and incorporates bioaugmentation to accomplish PCB degradation and dechlorination. For the aerobic degradation, field application vectors, FAVs, are used to avoid the need for the inducer, biphenyl. The FAVs are compatible with use of a relatively high surfactant concentration for soil washing. The surfactant solution fortuitously acts as a selective medium favoring growth of the FAVs over other aerobes. The FAVs contain the genes for aerobic PCB degradation. The process also incorporates the dechlorinating capacity from a river sediment for transforming higher chlorinated congeners to lower chlorinated forms. The individual operating steps of the process are connected in such a way as to allow repeated degradation and dechlorination cycles. Aerobic degradation following dechlorination exposes the lower chlorinated congeners formed by the dechlorination to further aerobic degradation by FAVs. Cycles of aerobic degradation and anaerobic dechlorination can be repeated as needed to obtain a desired level of PCB destruction.

Operation of the process in the field is envisioned to proceed as shown in Figure 3-1. PCB-contaminated soil is either isolated in a cell for processing or left in place in a partitioned sector of the substation. A 10,000 ppm surfactant solution is prepared in the wash tank. The surfactant solution is pumped over the soil at a designated rate to accomplish the solubilization of PCBs. Surfactant wash cycles are repeated as needed for efficient PCB recovery. When washing is complete, the PCB-containing solution is placed in a bioreactor along with diatomaceous earth, nutrient, acid and base as needed. An inoculum of *Ralstonia eutrophus* B30P4::TnPCB and *Pseudomonas putida* IPL5::TnPCB is added to the bioreactor. The bioreactor contents are aerated and agitated while surfactant is metabolized. When the surfactant concentration reaches a level below its CMC, certain PCB congeners are degraded by the FAVs. The residual, more highly-chlorinated PCB congeners are deposited on the diatomaceous earth as they become less solubilized due to disappearance of surfactant. The contents of the bioreactor are pumped to a centrifuge. Process water is recovered and pumped to a storage tank. The water is used as make-up water for subsequent aerobic degradation or for the preparation of wash solution to process another batch of contaminated soil. The PCBs on diatomaceous earth are moved to the anaerobic reactor. After a period of anaerobic incubation to dechlorinate the residual congeners, the diatomaceous earth is removed for disposal or sent back to the bioreactor for further aerobic degradation processing.

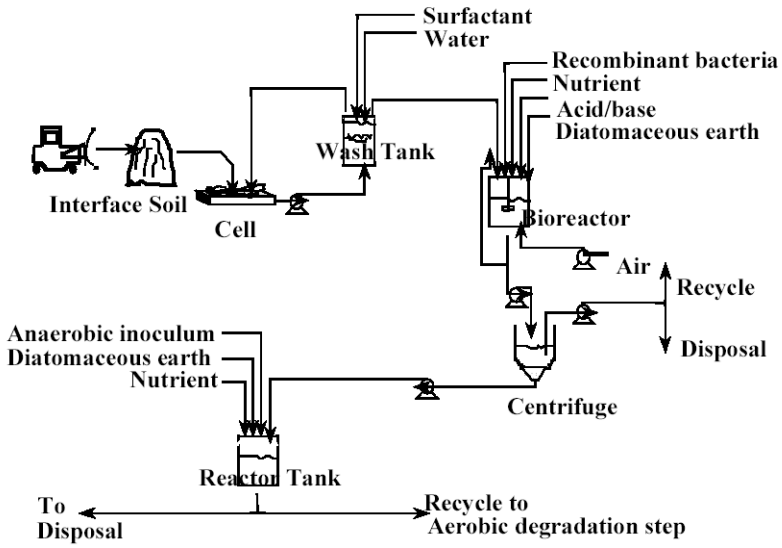

Figure 3-1
Conceptual Diagram of the In Situ Soil Irrigation Process for Biotransformation of PCBs

The process is based on these features for maximizing PCB cleanup. A surfactant-mediated soil wash can efficiently recover PCBs from soil. Aerobic microbial degradation in a bioreactor setting minimizes bioavailability problems associated with PCBs sorbed to soil. Alternating cycles of anaerobic dechlorination and aerobic degradation remove chlorines from highly chlorinated PCB congeners making these congeners susceptible to further aerobic degradation. Aerobic degradation is, of necessity, the first biologically mediated step of the in situ soil irrigation process because anaerobic dechlorination is sensitive to relatively high concentration surfactant used to recover PCBs. Once surfactant is metabolized in the first aerobic degradation cycle, anaerobic dechlorination is applied. Aerobic degradation proceeds by means of the FAVs, the recombinant bacteria. FAVs use surfactant as a growth substrate and at the same time degrade PCB congeners. Subsequent anaerobic processing further dechlorinates PCB congeners making them susceptible to additional aerobic degradation. Repeated cycles of alternating aerobic and anaerobic incubation can be applied to optimize PCB destruction.

Results of laboratory work were compiled to demonstrate efficiencies for the unit operations of the in situ soil irrigation process (Table 3-1). A single pass through the process can account for a laboratory scale efficiency of 56% PCB destruction.

Table 3-1
Process Efficiencies at Laboratory Scale of a Single Pass through the In Situ Soil Irrigation Process for PCB Biotransformation

Unit Operation at Laboratory Scale	Efficiency of Unit Operation
Soil Washing	0.75
Aerobic PCB degradation	0.35
Liquid-solids separation	0.90
Anaerobic PCB dechlorination	0.68
Total overall processing	0.56

Pilot Scale Test Design

Equipment requirements shown for the process concept (Figure 3-1) were modified to conduct a pilot scale test. Equipment allowing good scientific data and ensuring containment of the FAVs was selected. Demonstration equipment included a 60-L EIMCO biolift reactor (EIMCO Process Equipment, Salt Lake City, UT); an approximate 70-L sealed, stainless steel tank; and a 126-L stainless steel soil cell (Figure 3-2). The biolift reactor served alternately as the reservoir for preparing POL solution, as the aerobic degradation reactor, the storage vessel for process water, and ultimately as the anaerobic dechlorination reactor. The stainless steel tank served alternately as the storage vessel for process water and the anaerobic dechlorination reactor. A single pump served each pumping requirement. A centrifuge was unsuitable for the liquid-solid separation as discussed in this report and was omitted.

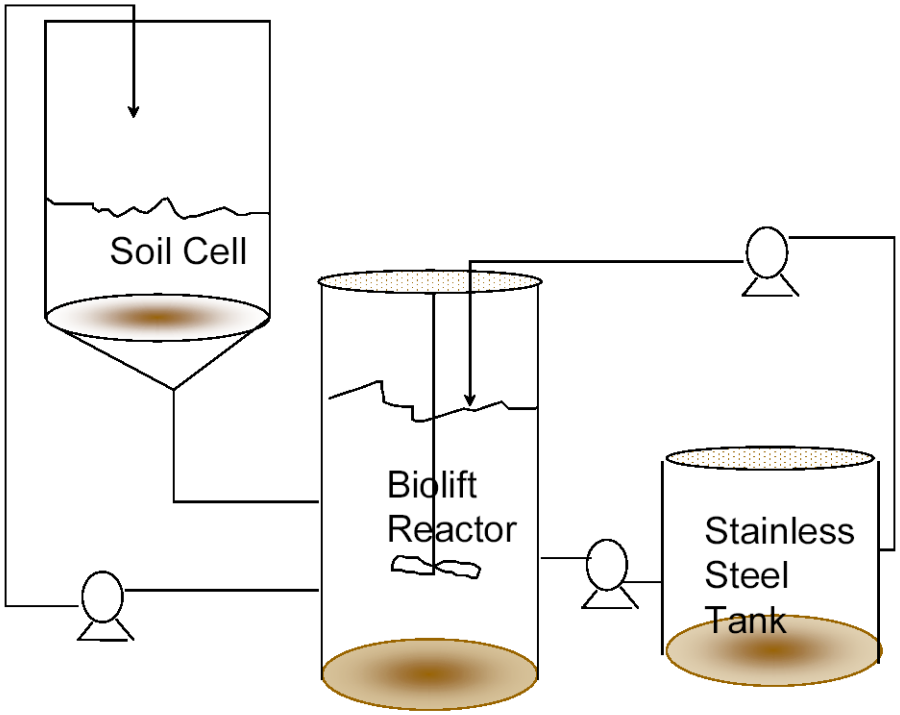

Figure 3-2
Pilot Scale Equipment Arrangement

A pH set point of 7.0 was established. A controller activated a pump to add 1N NaOH when the pH reading fell below 7.0 and 1N H_2SO_4 when the reading was above 7.0. The amounts of acid and base added were tracked by periodic weighing of tared respective acid and base reservoirs.

A dissolved oxygen (DO) set point of 3.0 mg/L was selected. By comparison, the DO reading of fully oxygenated water is about 8 mg/L. The signal from the DO probe was fed to a controller that turned on the in-house compressed air flow to reactor diffusers when the DO reading was 3.0 mg/L or less. This setting was a compromise aeration level intended to stimulate aerobic microbial growth yet minimize foaming. Although maintaining DO closer to oxygen saturation was desirable, the aeration required to maintain saturation would have triggered additional

foaming resulting in even larger amounts of antifoam added. The DO readings were recorded periodically throughout the aerobic operation.

A conductivity sensor was used in foam control. When foam in the reactor rose a few inches above the liquid contents and contacted the sensor, the controller started a pump to add a 1:10 solution of Dow 1510-US in tap water. Antifoam use was tracked by periodically weighing the tared antifoam reservoir.

An equipment modification was made in order to sample the biolift reactor. A pump and piping were installed to constantly recirculate reactor liquid contents through a 1-L stainless steel reservoir at a rate of 9.5 L/min. Liquid samples were withdrawn from the reservoir by means of a valve added to the bottom of the reservoir. The reservoir also held the DO and pH probes. The modification is depicted in Figure 3-3.

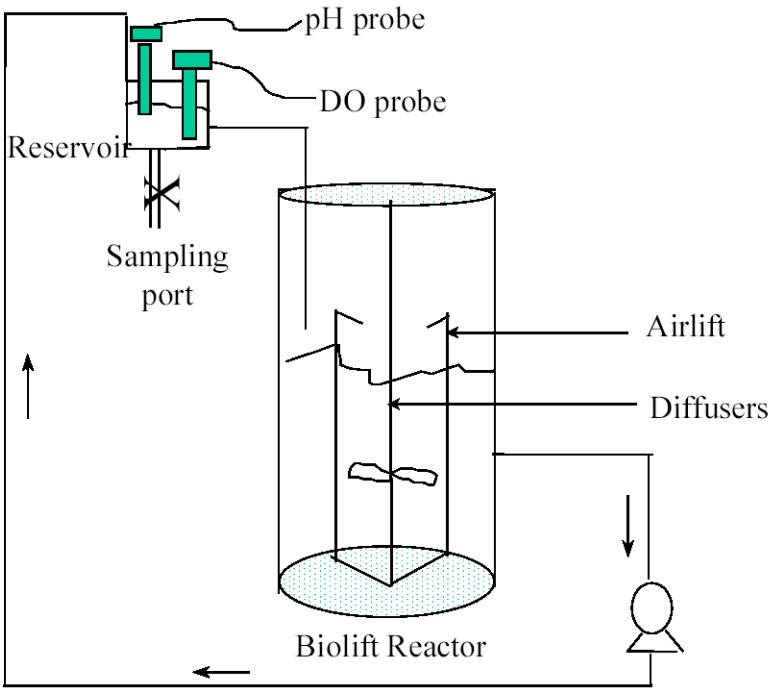

Figure 3-3
Biolift Reactor with Modifications for Control and Sampling

The diffuser air flow rate for the biolift reactor was arbitrarily set at a gauge reading of 50 std ft^3/hr. This reading is the equivalent of 1400 L/hour. Air flowed through the diffuser when required for maintaining the desired DO level. The central airlift mechanism, which both raked the bottom of the reactor and pumped liquid from the bottom for recirculation at the top of the reactor, was run continuously.

The soil cell was a cylindrical, stainless steel vessel approximately 46 cm in diameter and 76 cm deep. A convex, stainless steel screen covered the drain at the bottom, center of the vessel. A 50-kg portion of contaminated soil occupied an approximate 25 cm depth in the cell.

Shakedown Runs

Two shakedown runs were made of the biolift reactor prior to the pilot scale test. All process components, except PCBs from contaminated soil, were included. The details of the components are presented in later sections of the report. Components included 4.2 L of nutrient solutions, 250 g diatomaceous earth, 50 L of 10,000 ppm POL solution, and 1 L of inoculum consisting of *Pseudomonas putida* IPL5::TnPCB and *Ralstonia eutrophus* B30P4::TnPCB. These were introduced into the biolift reactor. The runs made the need for foam control apparent. A mechanical foam breaker could not control the production of excess foam.

To combat the foam, an antifoam was used. The antifoam selected, Dow 1510-US (Dow Corning, Midland, MI), is food grade antifoam. Its recommended concentration for food use is 100 ppm. A laboratory test of the effects of a similar antifoam, Antifoam B (Dow Corning) was performed. Dilutions of POL were prepared in sterile tubes and Antifoam B added to each at a constant 1000 ppm concentration. Growth of the FAVs was proportional to the POL concentration over the range 2000, 4000, 5000, and 10,000 ppm POL. These data indicate that Antifoam B, even at 1000 ppm, had no effect on POL metabolism by the FAVs. It is therefore assumed that Dow 1510-US at concentrations for pilot scale has no harmful effect on the FAVs.

Operations of the biolift reactor improved after the installation of foam and dissolved oxygen control utilizing respective antifoam addition and controlled agitation and aeration to maintain a DO set point of 3 mg/L.

General Conditions of Pilot Scale Operation

Tap water was used in all operations of the process. All operations were conducted at room temperature. Duplicate samples were taken for the analysis of liquid and solid components at all sampling points. The preparation of FAVs for inocula, anaerobic sediment inocula, and nutrient stock solutions and the chemical and biological sampling were performed under standard procedures for sterility in a microbiology laboratory. Operations such as the addition of diatomaceous earth, tap water, acid, base, surfactant and antifoam were performed under sanitary, but not sterile, conditions. All operations were conducted in a 270 m^3 room at TVA's Environmental Research Center, Muscle Shoals, Alabama. The room was equipped with an overhead ventilation system but had no special filters or hoods for microbiological studies. At times, the abrasive diatomaceous earth-containing slurry could not be transferred between vessels by means of a pump. At those times, a beaker was used to transfer solids and liquid between vessels. The soil cell, biolift reactor, and stainless steel tank were set inside plastic wading pools as a means of secondary containment against spills.

Prior to the demonstration, equipment and transfer lines were filled to capacity and flushed three or more times with a 1:100 dilution of Clorox in tap water followed by a tap water flush. This sanitizing procedure dislodged solids from the biolift reactor walls that consisted of diatomaceous earth deposited on the reactor walls during the previous shakedown runs.

Unit Operations at Pilot Scale

The unit operations were performed in sequence as indicated in the concept diagram (Figure 3-1). The solids from the anaerobic dechlorination were recombined with the

liquid from the final separation for an additional cycle of aerobic degradation, liquid-solids separation, anaerobic dechlorination, and final liquid-solids separation. Samples of the interface in the soil cell, liquid and solid components throughout the pilot scale test, the final liquid and solids streams, and the equipment to provide data for a materials balance.

Soil Wash

A 10,000 ppm POL wash solution was prepared by first making a concentrated solution of 500 g POL in 5 L of tap water. The mixture was heated in an oven at 105º C to dissolve the POL. The concentrated POL solution was transferred to the biolift reactor and diluted with tap water. The biolift reactor served as the mix tanks for the 10,000 ppm POL solution.

PCB-contaminated interface (50 kg) was placed in the soil cell. A grid sampling design was followed taking seven cores from the cell before and seven after soil washing. Initially a sprinkler was used to evenly distribute the 10,000 ppm POL solution over the interface surface. The interface became saturated rapidly so that the sprinkler proved to be unnecessary and its use was discontinued. The entire 50 L of wash solution was added in 10 minutes in order to have the solution pool on the interface surface. In 50 minutes the solution had drained from the interface by gravity into the biolift reactor. The same POL solution was repeatedly pumped onto the interface in 10 min cycles and subsequently allowed to drain for 50 minutes. These wash-drain cycles were repeated continuously for two days. At the water holding capacity for interface of 100 g water per kg soil, each 50 L of POL solution pumped over the interface is the equivalent of 10 pore volumes.

A materials balance was determined for the soil wash processing step using two calculations. The two calculations were in good agreement. The first calculation relied on the wash solution PCB analysis. It was found that 1.4 kg of wash solution was held up in the interface as determined by wet weights of the interface before and after wash. This holdup solution was assumed to have the same PCB concentration as the wash solution. The PCBs recovered in liquid were determined as the sum of PCBs in wash solution and holdup solution. A second calculation of wash efficiency was based on the difference in PCBs in the interface before and after the wash. By the first calculation, PCBs in solution account for 50% of the PCBs in the original interface. By the second calculation, 51% of the PCBs were removed from the interface (Table 3-2).

Table 3-2
Data Sources for Calculation of Soil Washing Efficiency

Data sources for Calculations of PCBs Recovered by Soil Washing	Wash Efficiency (%)
Calculation based on PCBs recovered • PCB analysis of interface for testing • PCB analysis of wash solution • Calculation of PCBs contained in solution held up by interface	50
Calculation based on PCBs removed • PCB analysis of interface before washing • PCB analysis of interface after wash cycle	51

At laboratory scale and using the repeated wash-drain cycles for washing a kilogram of interface, 75% of PCBs in interface were recovered (Beck et al. 1996). However, at laboratory scale, about 10% of the liquid was lost due to foaming. At pilot scale, a good accounting of the wash solution was made. At pilot scale, the PCB-containing wash solution was partitioned in the interface and in recovered liquid. Together these two sources of wash solution accounted for 97% of the original wash solution volume. The interface held up 17% of the POL used for solution preparation, and 83% of the POL was in the wash solution at the end of two days of soil washing (Beck 1998). It does not seem likely that a final water wash to recover POL from the interface would have netted a substantial gain in PCB recovery. Such an effort would have further diluted this PCB-containing solution for the aerobic degradation cycle to follow. Because the interface was previously determined to be practically devoid of PCB-degradative microbes, residual POL is unlikely to promote further PCB degradation if the interface is stored in the soil cell for any length of time.

It is difficult to compare the pilot scale soil washing results with results reported in the literature. Whereas one large volume of surfactant was recycled repeatedly over interface containing 146 ppm weathered PCBs in this pilot test, Abdul and Ang (1994) added fresh volumes of surfactant to interface in a field test with PCB concentrations ranging up to 6000 ppm and oil up to 67,000 ppm. Likely their high PCB and oil contamination levels indicate free contaminant not sorbed to soil. They report that 25% of the PCBs and 32% of the oil were recovered after only 8 washings or pore volumes. By contrast, the pilot scale washing was the equivalent of about 480 pore volumes to remove 51% of the PCBs in the interface. They plotted PCBs remaining in soil versus pore volumes of wash. Their rate of recovering PCBs was quite good until the percent PCB remaining in interface dropped below 30%. At that point, the slope of the extraction curve declined significantly. Thus, after the PCB concentration fell below about 1800 ppm PCB, many more pore volumes of wash were required for recovering comparable amounts of PCB. Their results support a conclusion that relatively low concentrations of PCBs, such as the 146 ppm in the substation interface, require extensive washing for PCB removal.

On the other hand, Sheets et al. (1999), using a soil washing apparatus delivering 60 L of surfactant and pH-adjusted water wash to 2 kg soil, achieved respective 71, 95, and 69% recoveries from soil with 52, 140 and 700 ppm PCBs. Extraction of PCBs in more complicated systems, such as GHEA Associates process, New Jersey Institute of Technology, have demonstrated that PCBs can be extracted at rather high efficiencies. The reported an efficiency as high as 99.8% for a clayey soil, but provided little detail of how this was achieved (Environmental Protection Agency 1994).

The soil wash is pivotal for the overall success of the in situ soil irrigation process. Even if subsequent processing steps are highly efficient, the relatively low efficiency of the PCB recovery from interface limits the process efficiency. This step must be greatly improved if the in situ soil irrigation process is to be successful.

Aerobic Degradation

To the PCB-containing wash solution in the biolift reactor were added PAS salts and PA concentrate (Bedard et al. 1986), yeast extract, diatomaceous earth, tetracycline, *Ralstonia*

eutrophus B30P4::TnPCB and *Pseudomonas putida* IPL5::TnPCB. The amounts of each are given in Table 3-3. Agitation and aeration were employed to maintain the 3.0 mg/L DO set point. Acid and base additions maintained a pH of 7. Foam was monitored and controlled. Microbial populations were monitored.

Table 3-3
Biolift Reactor Components for First Aerobic Degradation Cycle of Pilot Scale Test

Component	Amount
Soil wash	48.4 L
PAS salts solution	0.5 L
PA Concentrate	3.75 L
Yeast extract, 5%	0.05 L
Diatomaceous earth	250 g
Ralstonia eutrophus B30P4::TnPCB	0.5 L
Pseudomonas putida IPL5::TnPCB	0.5 L
Tetracycline	0.625 g

The microbial inoculum of *Ralstonia eutrophus* B30P4::TnPCB and *Pseudomonas putida* IPL5::TnPCB was prepared in the TVA laboratories from source strains supplied by UT. Source strains were inoculated on solid PAS medium containing 0.2% POL plus 12.5 µg/ml tetracycline. After solid medium was incubated overnight at 37º C, colonies were picked to inoculate sterile tubes of PAS broth. The broth cultures were incubated in a similar manner. Sterile glycerol was added to each tube for a 15% glycerol concentration, and tubes were frozen at -70º C until used to inoculate the biolift reactor to an approximate density of 10^6 bacteria/ml.

Over the course of the aerobic degradation cycle, the bacterial counts were the same whether the plates contained biphenyl or not. The counting method did not distinguish a biphenyl-degrading bacterial population as a subset of the total population. Figure 3-4 follows the course of the total heterotroph population, the DO readings, the POL concentration, and the cumulative 1 N NaOH additions. The recombinant bacteria used 68% of the POL during the aerobic cycle. The residual POL concentration at 9 days was just over 2000 ppm. Over 1000 ml of 1 N NaOH maintained pH 7 to apparently neutralize acid formed due to POL metabolism. The DO reading dropped steadily from 9 to 3 mg/L during the early part of the degradation cycle reflecting oxygen demand of the increasing bacterial population. The DO reading subsequently rose to about 6 mg/L during the latter portion of the cycle as the population began to decline. The controller called for antifoam during the first half of the aerobic degradation cycle when the DO reading fell and aeration was demanded (data not shown). For example, after two days of incubation, the antifoam added was the equivalent to produce a concentration of 70 ppm. By the end of the incubation, the equivalent antifoam concentration would have been 180 ppm.

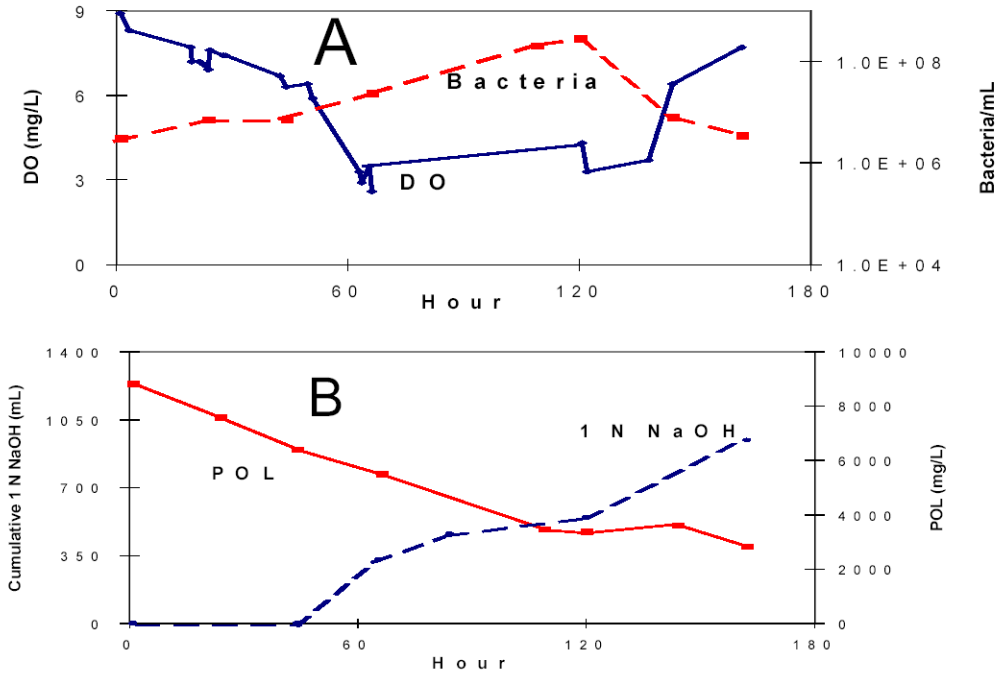

Figure 3-4
Time Course of the First Aerobic Degradation Cycle Including the (A) Bacterial Population, DO Readings, (B) POL Concentration, and Cumulative NaOH addition

The effects of implementing foam control can be inferred from a comparison of the pilot scale outcome to laboratory scale testing without foam control. Layton (1995) described a laboratory scale trial of the aerobic degradation of PCBs in POL solution that included the recombinant bacteria containing only the tetracycline genes (*Pseudomonas putida* IPL5::TnTC and *Ralstonia eutrophus* B30P4::TnTC). At laboratory scale, DO was not monitored nor control of oxygen level implemented. Air was used in sparging the reactor vessel at the beginning of the incubation, but oxygen was soon substituted for air to support bacterial growth. There was no foam control, and as a result, over 20% of the vessel contents were lost as spillover occurred due to foaming. The bacterial population had increased from 10^7 to 10^{10} bacteria/ml between 0 hour and 2 days at the expense of the spillover. By contrast, DO was monitored, oxygen in the liquid controlled, and foaming controlled by addition of antifoam at pilot scale. The recombinant bacterial population at pilot scale increased from 10^6 to 10^8 bacteria/ml in 4.5 days. At laboratory scale, residual POL solution was less than 5 ppm after 8 days of incubation (Beck et al 1996). The residual POL concentration exiting the pilot scale aerobic cycle was 2842 ppm. The contrast between 68% microbial degradation of POL at pilot scale and the 94% POL degradation at laboratory scale may be due to a negative impact of the antifoam on bacterial growth. Foam control at pilot scale likely resulted in less oxygen for bacterial degradation in comparison to the laboratory scale test without foam control. However, the spillover experienced at laboratory scale emphasizes the need to avoid such events both from a processing and a biological containment standpoint. Balancing oxygen demand and foam control likely prohibits maximum microbial activity rates.

As the subsequent analyses of the solids and liquid exiting the biolift reactor indicate, there was a 30% degradation of PCBs during aerobic degradation, cycle 1. A 35% degradation had been achieved at laboratory scale (Beck et al. 1996). The pilot scale outcome is in relatively good agreement with laboratory test results. Figure 3-5 illustrates the changes in PCB congener-containing chromatographic peaks resulting from the aerobic degradation cycle. Figure 3-5 A is the PCB pattern of the wash solution before degradation. Figure 3-5 B is the chromatographic patterns of PCBs in the liquid phase and PCBs associated with the solids at the end of degradation. The congener-containing pattern of the exiting liquid and solids were practically the same. This suggests that diatomaceous earth does not selectively sorb certain congeners and not others. Those chromatographic peaks that show the most change over the course of the aerobic degradation represent congeners of 3 and 4 chlorines. There was a trend toward the lower and midrange peaks decreasing in concentration over time. This reflects degradation of these congener. Such a pattern of congener loss is consistent with microbial aerobic degradation.

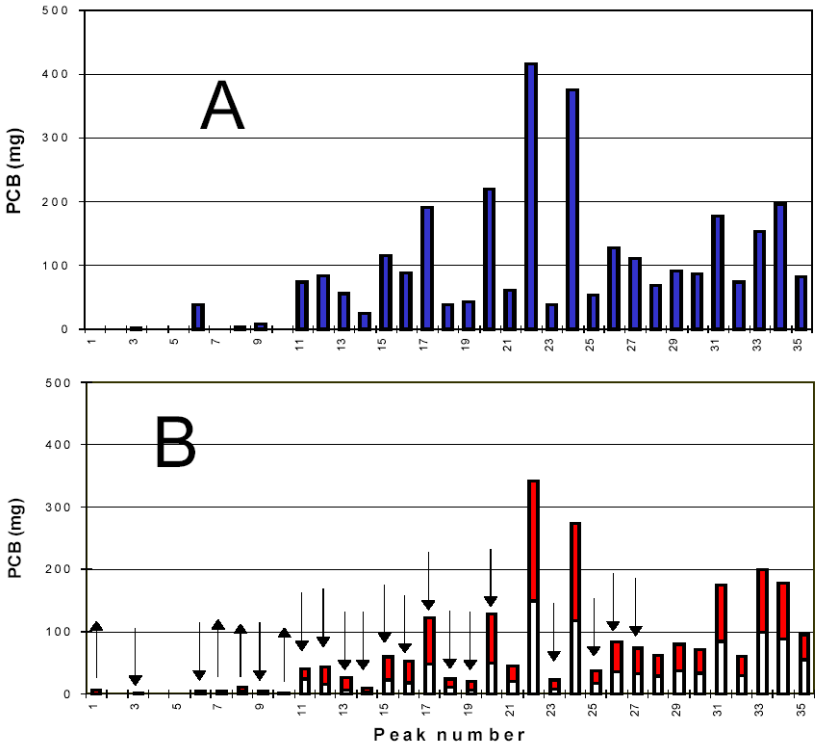

Figure 3-5
Changes in PCBs during the First Aerobic Degradation Cycle as Illustrated by (A) the PCB Pattern of Entering POL Wash Solution and (B) Exit Liquid (Solid Bar) and Solids (Open Bar) After Degradation. Arrows Indicate Chromatographic Peaks that Changed in Value by 30% or more over the Time Course. Up Arrows Indicate Increases and Down Arrows Decreases in Congener Amounts

After the first aerobic degradation cycle, the liquid and solids were separated, and the first anaerobic dechlorination was performed. The outcomes of these operations are described in the following sections of the report. After anaerobic dechlorination, the contents of the stainless steel tank and the stored process water were recombined in the biolift reactor for a second aerobic degradation cycle. The conditions of aeration, agitation, foam control, and monitoring that were applied for the first aerobic degradation were reinstated for the second aerobic degradation.

A predetermined amount of POL, intended to bring the concentration in the biolift reactor to 10,000 ppm POL, was added at 0 hour of the second aerobic degradation. This predetermined amount of POL was based on the assumption that the POL concentration of the recombined streams should be 2000 ppm. It was assumed that there was no degradation of POL in process water or in the anaerobic dechlorination. After adding the predetermined amount of makeup POL, the resulting POL concentration was 6709 ppm and accounted for the added POL only. Therefore, it was concluded that POL was consumed during the anaerobic dechlorination cycle but not in the process water. Unfortunately, the time course of POL consumption was not tracked in the anaerobic dechlorination cycle.

During the course of this second aerobic degradation, 69% of the POL was used by the recombinant bacteria. The exit POL concentration was 2102 ppm, similar to the exit POL concentration during the first aerobic degradation. However, the time course of changes in DO readings and bacterial counts differs from the first aerobic cycle. The DO readings dropped in response to rapid bacterial growth earlier in the time course and returned to mid-range more rapidly than in the first aerobic degradation. As in the first aerobic degradation, the bacterial counts on media with and without biphenyl were practically the same. The bacterial population reached 10^7 bacteria/ml by 20 hours. However, there was no additional increase in numbers after 20 hours as in the first aerobic degradation. Figure 3-6 depicts the time course of the second aerobic degradation.

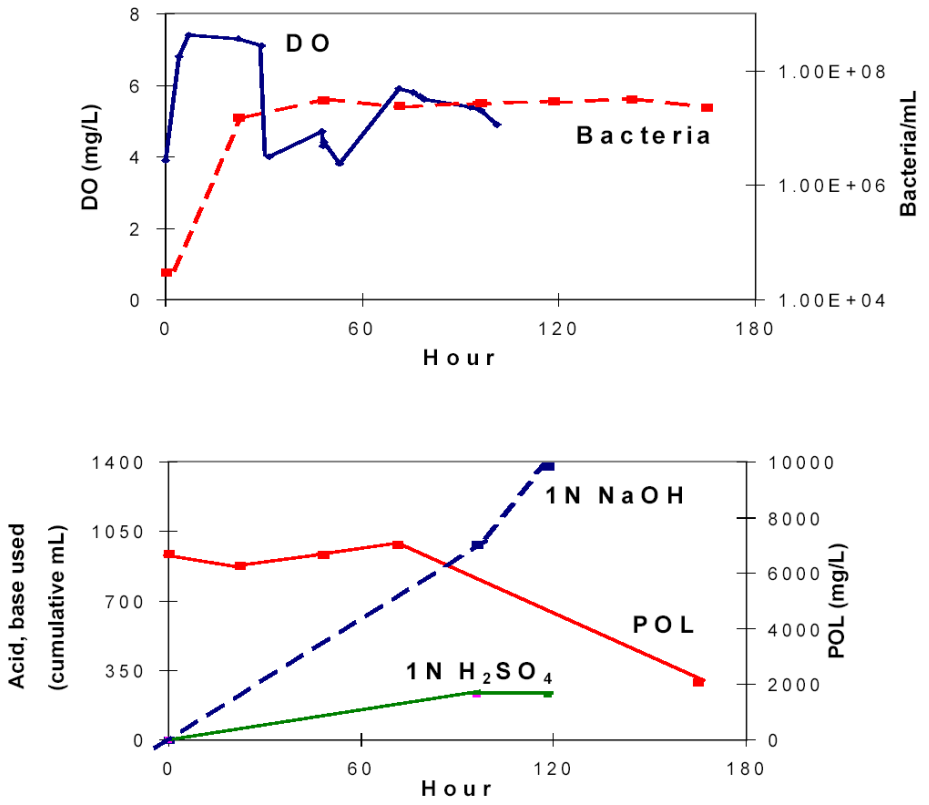

Figure 3-6
Time Course of the Second Aerobic Degradation Cycle Including (A) The Bacterial Population, DO Readings, (B) POL Concentration, and Cumulative 1 N NaOH and 1 N H_2SO_4 Additions

During the second aerobic degradation cycle a spillover occurred. The spillover was due to the peristaltic pump pulling the end of the tubing above the surface of the antifoam supply. When antifoam was called for, none reached the biolift reactor. Before the problem was discovered, about 5 L of reactor contents foamed into the containment reservoir.

The pH was maintained about 7.0 during the time course. The pH control differed from that experienced during the first aerobic degradation. Both 1 N NaOH and 1 N H_2SO_4 were called for by the controller to maintain pH 7.0. It is not known what types of perturbations caused the controller to call for 1 N H_2SO_4. In the first aerobic cycle and during shakedown runs, only 1 N NaOH was called for in pH control. The amount of 1 N H_2SO_4 called for in the second aerobic degradation accounts for the neutralization of 459 ml of 1 N NaOH. Therefore, the balance of 1 N NaOH added to the reactor was 923 ml. The 1 N NaOH called for during the first aerobic degradation was 944 ml. The closeness in the two amounts of NaOH suggests similar microbial activity for both aerobic cycles. However, PCB degradation was much less in the second than in the first aerobic degradation. The entering PCB concentration for the second aerobic degradation was 1936 ppm, and the exiting concentration was 1753 ppm PCB. Thus, 9% of the PCBs entering were degraded during the second aerobic degradation.

Almost three times as much antifoam was called for in the second aerobic degradation as in the first (data not shown). By the end of the incubation, antifoam addition accounted for an antifoam concentration of 490 ppm. This was in contrast to a concentration of 180 ppm over the first aerobic degradation. It is possible that the increased amount of antifoam over the course of the second aerobic degradation diminished bacterial growth and PCB metabolism. Also the swings of pH that called for both acid and base additions may have had a negative effect on growth and PCB metabolism.

Figure 3-7 contains a comparison of the PCB congener-containing chromatographic peaks entering and exiting the second aerobic degradation. Fewer of the lower chlorinated PCB congener-containing chromatographic peaks decreased in concentration over the time course in comparison to the first aerobic degradation. Because of the unusual events of this second aerobic degradation, the real value of repeated cycles of aerobic degradation and anaerobic dechlorination for improving the overall PCB destruction was not demonstrated.

The efficiencies of these two aerobic degradation cycles fit into the overall materials balance. Table 3-4 includes the degradation efficiencies of each of the two aerobic degradation cycles.

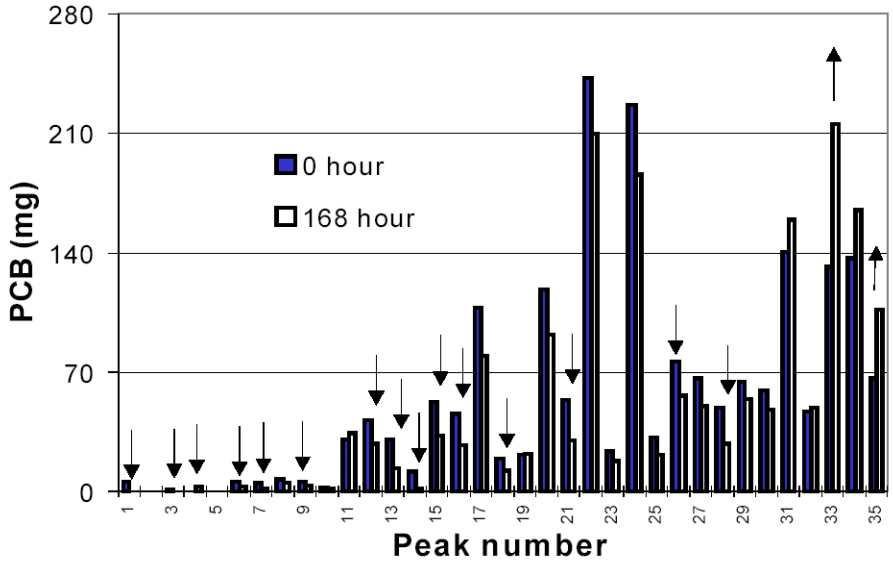

Figure 3-7
Comparison of Entering and Exiting Congener-Containing Chromatographic Patterns in the Second Aerobic Cycle. Arrows Indicate Chromatographic Peaks Increasing or Decreasing 30% or More Over the Time Course. Up Arrows Indicate Increase; Down Arrows Indicate Decrease.

Table 3-4
Efficiency of Aerobic Degradation Cycles

	PCBs Entering Cycle (mg)	PCBs exiting Cycle (mg)	PCBs Degraded during Cycle (mg)	Percentage of the total PCBs Degraded
First aerobic degradation	3103	2176	927	30
Second aerobic degradation	1936	1753	183	6

Liquid-Solids Separation

For pilot scale liquid-solids separation, a settling process was used. As discussed elsewhere in this report, the use of centrifugation was ruled out. Aeration and agitation were stopped at the end of aerobic degradation cycle, and the biolift reactor contents were allowed to settle for a day. After the reactor contents settled, the bottom portion of the reactor contents was pumped to the stainless steel tank for anaerobic dechlorination. Thus the diatomaceous earth arrived in the stainless steel tank as a slurry. A tare weight of the stainless steel tank and its weight upon receiving the solids were used in determining the materials balance. The supernatant liquid or process water remained in the biolift reactor to later be recombined with solids from the stainless steel tank for the second aerobic degradation. It was obvious that for the second aerobic degradation and subsequent liquid-solids separation an more effective method may be to pump the supernatant liquid to the stainless steel tank for storage and leave the solids in the biolift

reactor for anaerobic degradation. This change in operation requires much attention to detail to have the biolift reactor serve as an anaerobic reactor.

Diatomaceous earth was added to the aerobic degradation for the purpose of sequestering PCBs and moving them forward to anaerobic dechlorination. It was hypothesized that total PCBs can be captured in this way. In reality, the PCBs associated with diatomaceous earth and a small amount of accompanying liquid accounted for 39% of the PCBs emerging from the liquid-solids separation. The balance, or 61% of the PCBs, remained in the process water. An efficiency of 90% recovery of PCBs with the solids was achieved for a laboratory scale test liquid-solids separation (Beck et al. 1996). At laboratory scale the final POL concentration was well below the CMC. At pilot scale, the aerobic degradation exit POL concentration was 2842 ppm. It does not appear that most PCBs moved to the anaerobic cycle on either biomass or diatomaceous earth. Instead some PCBs were apparently carried in the surfactant micelles. About 81% of the aerobic cycle liquid went forward as process water and 19% was incorporated with the slurry passing to the stainless steel tank. Apparently a great portion of the PCB congeners simply moved in the micelles.

Bacterial counts of the process water and the solids in the stainless steel tank were made. The stainless steel tank was purged with nitrogen in preparation for anaerobic dechlorination prior to collecting samples for the bacterial counts. Only 3% of the bacterial population from aerobic degradation was accounted for in the process water and another 3% in the stainless steel tank. In theory, the bulk of the biomass should have settled with the diatomaceous earth and moved forward to the stainless steel tank. Perhaps because anaerobic conditions were imposed prior to withdrawing the bacterial count sample from the stainless steel tank, a large heterotroph population was not found. The population may have become nonviable between the end of the aerobic cycle and after the point of imposing anaerobic conditions in the stainless steel tank.

A second liquid-solids separation was performed after the second aerobic degradation cycle. Solids were allowed to settle in the biolift reactor, and process water was decanted to the stainless steel tank. About 58% of the biolift reactor liquid contents were pumped as process water and 42% of the liquid left with the anaerobic dechlorination slurry in the biolift reactor. The water distribution parallels the PCB distribution. About 45% of the PCBs were found in the process water and 55% in the anaerobic slurry. As with the first liquid-solids separation, much of the PCB was apparently carried in the surfactant micelles. Liquid-solids separation efficiencies are shown in Table 3-5.

Table 3-5
Efficiency of Liquid-Solids Separation Steps

	Liquid forward (mg PCBs)	Solids forward (mg PCBs)	Efficiency of Liquid-Solids separation (percentage PCBs forward with solids)
First liquid-solids separation	1323	853	39
Second liquid-solids separation	652	803	55

Anaerobic Dechlorination

Sequential anaerobic-aerobic schemes have been proposed to take advantage of the better attack of more highly chlorinated biphenyls by anaerobes, in comparison to aerobes, and the ability of aerobes to oxidize the less chlorinated biphenyls (Abramowicz 1990). The in situ soil irrigation process capitalizes on this attribute as well.

The PCB-containing solids slurry from the liquid-solids separation was pumped into the stainless steel tank for the anaerobic dechlorination. Sufficient diatomaceous earth was added to the 10.3 kg slurry to attain a calculated concentration of 1000 ppm PCBs on diatomaceous earth. The actual amount of the PCBs in the tank resulted in a concentration of PCBs on diatomaceous earth of just over 400 ppm. The stainless steel tank was also supplied with 0.6 L anaerobic inoculum and 1 L of 10X nutrient solution containing resazurin. The inoculum was a preincubated, sediment-free filtrate from a river sediment of known anaerobic degrading capacity. Mark Reeves and John Barton, ORNL, prepared the inoculum and nutrient solution as described elsewhere (Klasson et al. 1996). They set an inoculum rate on a weight basis of about one part inoculum to 30 parts diatomaceous earth. No other compounds were added to induce or prime dechlorination.

The tank was purged with nitrogen for over five minutes at the beginning of the cycle and each time the anaerobic dechlorination was sampled over nine weeks. The slurry color verified that the reactor contents were anaerobic at each sampling. The anaerobic dechlorination was sampled through ports near the bottom of the stainless steel tank. No anaerobic microbial analyses of the inoculum or any samples over the incubation period were performed. Counts were made to determine a total population and a population growing in the presence of biphenyl. The counts for the two populations were practically the same. Counts of 5×10^5 bacteria/ml at 0 hour, 1×10^5 at 4 days, and 6×10^4 were obtained at 9 weeks. The purpose of the counts during the anaerobic incubation was to check the survival of the recombinant bacteria. Because the populations growing aerobically on media with and without biphenyl were practically the same in number, it is assumed that the total population count is a count of the FAVs. The identification of the bacteria was not confirmed.

The POL concentration at the outset of the anaerobic dechlorination was 2842 ppm. As described previously in this report, there is indirect evidence this POL was completely utilized during the cycle.

Figure 3-8 contains a comparison of the congener-containing chromatographic peaks at the beginning and the end of the first anaerobic incubation. A number of samplings made over the time course support the trends of beginning and ending data. An attempt was made to discern a specific dechlorination pattern based on general trends as described by Bedard and Quensen (1995). There were no apparent trends such as preponderance of attack on congeners with either 2, 3 or 3, 4 chlorination. There was a general trend toward a decrease in the higher numbered peaks and an increase in lower numbered peaks. There was an overall loss of 28% of the PCBs during the anaerobic dechlorination. A 68% PCB reduction at 8 weeks was reported in a laboratory scale test (Beck et al. 1996). One possible reason for the lesser dechlorination at pilot scale as compared to laboratory scale is the presence of excess POL.

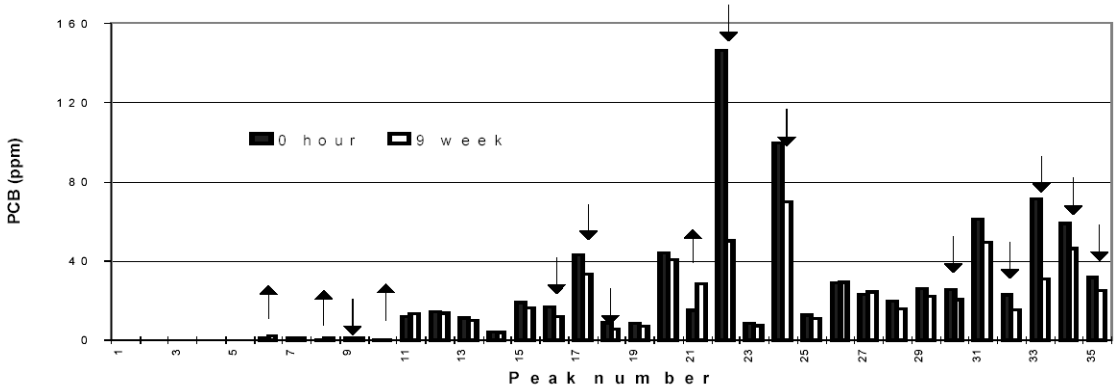

Figure 3-8
Comparison of 0 and 9 Week Congener-Containing Chromatographic Patterns in the First Anaerobic Cycle. Arrows Indicate Chromatographic Peaks that Changed by 20% or More Over the Course of Anaerobic Dechlorination. Up Arrows Indicate Increase; Down Arrows Indicate Decrease.

For the second anaerobic dechlorination, 1 L of nutrient solution and 0.5 L of the ORNL anaerobic inoculum were added. The inoculum was part of the original inoculum batch prepared by Barton and Reeves and was stored in the refrigerator about ten weeks before use. The rate of anaerobic inoculation was one part inoculum to ten parts slurry.

The conditions of the incubation were the same as for the first anaerobic dechlorination. The POL concentration was about 2000 ppm at the outset of the second anaerobic dechlorination. PCB analyses indicated no net gain or loss in total PCB congener-containing chromatographic peaks during this cycle. There was no change in the amount of POL entering and exiting the second anaerobic cycle. Operations were ceased at this point, materials recovered and accounted for, equipment cleaned, wastes sent to disposal, and analyses completed. Aspects of closure are described further in this report.

Materials Balance

A process materials balance was developed by determining both the overall outcome and the outcomes of individual processing steps. A variety of measurements and analyses were used in the calculations.

Process efficiencies were calculated by three means. For the first means, a balance sheet was kept throughout processing beginning with the soil washing of the 50 kg of interface and ending with final disposal of all PCB-containing materials. The balance sheet took into account sample withdrawals, various additions, and the spillover. Periodic PCB analyses and tracking of weights and volumes of process components aided this calculation. Table 3-6 is a summary of the data. Using the balance sheet, 80% of PCBs in the original interface are accounted for at the final disposal. The soil wash efficiency was only 50% efficient, resulting in half the PCBs never being exposed to biotransformation processing steps. Biotransformation processing and related steps were determined to be 40% efficient by this balance sheet method.

Table 3-6
Summary of the Balance Sheet of Fate of PCBs throughout the Pilot Scale Test

Description of Process Step and Activity	Volume (L) or Weight (kg) of Component	Cumulative Volume (L) or Weight (kg)	PCBs (mg) in Component	PCBs (mg) at end of Process Step
Interface before wash	47.7 (dry)	6964		
Samples	0.152	22		
Interface after wash	47.6 (dry)	3380		
Samples	0.126	9		
Subtotal				3371
First aerobic cycle 0 hour	48.2		3103	
Inoculum, nutrient, etc.	5.30			
Samples	0.28		16	
Diatomaceous earth	0.25			
Subtotal		53.47		3087
First aerobic cycle 168 hours	53.47			
PCBs in liquid			1277	
PCBs on solids			3073	
Samples	0.04		1	
Antifoam	0.1			
NaOH	0.94			
Subtotal		54.47		4349
Solids for first anaerobic dechlorination	10.3			
PCBs with liquid			175	
PCBs with solids			674	
Samples	0.25		8	
Diatomaceous earth	2.30			
Subtotal		12.35		841
Process water first liquid-solids separation	44.17		1318	
Samples	0.76		13	
Subtotal		43.41		1305
First anaerobic dechlorination, 0 hour	12.35			
PCBs with liquid			141	
PCBs with solids			896	
Samples	0.20		5	
Inoculum, nutrients	1.5			
Diatomaceous earth	0.10			
Subtotal		13.75		1032
First anaerobic cycle 2 weeks	13.75			
PCBs with liquid			171	
PCBs with solids			966	
Samples	0.30		22	
Subtotal		13.45		1115
First anaerobic cycle 4 weeks	13.45			
PCBs with liquid			167	
PCBs with solids			449	
Samples	0.23		5	
Subtotal		13.22		610

Table 3-6
Summary of the Balance Sheet of Fate of PCBs throughout the Pilot Scale Test (Continued)

First anaerobic cycle 6 weeks	13.22			
PCBs with liquid			185	
PCBs with solids			651	
Samples	0.21		6	
Subtotal		13.01		830
First anaerobic cycle 8 weeks	13.01			
PCBs with liquid			246	
PCBs with solids			281	
Samples	0.24		7	
Subtotal		12.76		520
First anaerobic cycle 9 weeks	12.76			
PCBs with liquid			178	
PCBs with solids			436	
Samples	0.2		5	
Subtotal		12.56		609
Second aerobic cycle 0 hour	55.97			
PCBs with liquid			2939	
PCBs with solids			87	
POL	1.00			
Inoculum, nutrients	5.30			
Samples	0.29		14	
Subtotal		61.99		3012
Second aerobic cycle 168 hour	61.99			
PCBs with liquid			1254	
PCBs with solids			436	
Samples	0.05		1	
NaOH	1.38			
Antifoam	0.30			
H_2SO_4	0.23			
Spill	5		105	
Subtotal		58.85		1583
Second process water	34.30		652	
Samples	0.27		5	
Subtotal		34.03		647
Second anaerobic cycle 0 hour	24.83			
PCBs with liquid			267	
PCBs with solids			536	
Inoculum, nutrient	1.50			
Samples	0.22		10	
Subtotal		26.11		794
Second anaerobic cycle 2 weeks	26.11			
PCBs with liquid			330	
PCBs with solids			725	
Samples	0.19		3	
Subtotal		25.92		1053

Table 3-6
Summary of the Balance Sheet of Fate of PCBs throughout the Pilot Scale Test (Continued)

Second anaerobic cycle 4 weeks	25.92			
PCBs with liquid			234	
PCBs with solids			866	
Samples	0.20		3	
Subtotal		25.72		1097
Second anaerobic cycle 6 weeks	25.72			
PCBs with liquid			278	
PCBs with solids			368	
Samples	0.19		2	
Subtotal		25.53		644
Second anaerobic cycle 8 weeks	25.53			
PCBs with liquid			690	
PCBs with solids			282	
Samples	0.17		7	
Subtotal		25.35		965

The efficiency of the biotransformation-related processing steps alone were determined by calculating each unit operation's efficiency, assuming no changes in weight or volume during the unit operation. Table 3-7 shows the efficiencies calculated for the individual processing steps related to biotransformation.

Table 3-7
Efficiency of Biotransformation-Related Processing Steps Alone

Processing Step	Efficiency of Processing Step (%)	Cumulative loss of PCB (%)
First aerobic cycle (biotransformation)	30	30
First liquid-solids separation (recovery on solids)	39	
First anaerobic cycle (biotransformation)	28	38
Recombining of process water and anaerobic cycle residue	100	
Second aerobic cycle (biotransformation)	9	43
Second liquid-solids separation (recovery on solids)	28	
Second anaerobic cycle (biotransformation)	0	

Ultimately, biotransformation-related process efficiencies were determined as the difference between PCBs in at the first introduction of wash solution to the biolift reactor and PCBs out. The PCBs out were found in final liquid and solids streams and in solids scraped from the sides of the vessels and hexane washes of the vessel walls. An allowance was made for the loss of PCBs in the approximate 5-liter spillover during the second aerobic cycle. By this method, the biotransformation-related processes were determined to be 43% efficient. Table 3-8 shows PCBs in and out of the biotransformation-related processing steps.

Table 3-8
Data used in Calculating a PCB Biotransformation Efficiency of 43% Based on Difference between PCBs in and PCBs out in Final Process Cleanup

	Components evaluated	PCBs (mg)
Input	PCBs entering bioprocessing	3103
Recovered	Diatomaceous earth slurry	89
	Solids off vessel walls	242
	Washes of vessel walls	560
	Process water	647
	Removed in 34 samples of various types	132
	Spill	105

The efficiency of the overall process is affected by a variety of operations. The soil washing step is crucial to the success and the overall efficiency of the in situ soil irrigation process for biotransformation of PCBs in soils. The PCB biotransformation and associated operations are only effective on those PCBs available for processing. The soil wash made only half the interface PCBs available to the biotransformation and associated operations. The 50% soil wash efficiency combines with the 43% biotransformation efficiency for an overall PCB reduction of 22% at pilot scale.

4
CONCLUSIONS

Plans for a field demonstration of the in situ soil irrigation process for PCB biotransformation in soils were halted due to the relative inefficiency of the process at pilot scale. The PCB-contaminated gravel, interface, and soil were provided proper disposal. The research site at Moccasin Bend was restored to use.

Disposition of Research Site

A notification and clean-up plan for the four capacitor bank areas was submitted to EPA Region 4 on February 5, 1999. Although the project sampling and analysis plan used in the project's characterization of the capacitor banks at Moccasin Bend contained a sampling grid unlike that specified by EPA for PCB clean-up (Environmental Protection Agency 1998), the project characterization was accepted as defining the PCB contamination. In fact, the project sampling grid provided more sampling points than the EPA plan. After excavation and disposal of PCB-contaminated soil at Moccasin Bend, a follow up sampling and analysis was performed. The sampling and analysis verified that PCB concentrations were less than 3 ppm in the underlying soil at the substation. The cleanup was completed on May 6, 1999.

TVA's Environmental Chemistry Laboratory in Chattanooga performed the follow up PCB analyses. They reported the residual PCBs as Aroclor 1254. Gross examination of the chromatographs of each sample in comparison to those of Aroclor 1242, 1248, and 1254 standards revealed a pattern of absence of the lower congeners of both 1242 and 1248. In addition, at least 5 peaks representing the most highly chlorinated congeners of 1254 were missing in the substation PCB profile. It was not possible to discern whether any additional change in the congener pattern of the residual PCB had occurred over the duration of the project life. The follow up analysis confirmed that PCBs had not migrated below the zone of contamination delineated in the project site characterization.

Implication of Laboratory and Pilot Scale Findings for In Situ PCB Remediation

The efficiencies of processing steps at laboratory and pilot scale are compared in Figure 4-1. The overall efficiency of 56% degradation for laboratory scale was superior to the 21% degradation efficiency at pilot scale. The pilot scale had incorporated a second aerobic degradation and a second anaerobic dechlorination. A comparison of the process operation outcomes at laboratory and pilot scale makes obvious the targets for improving process efficiency.

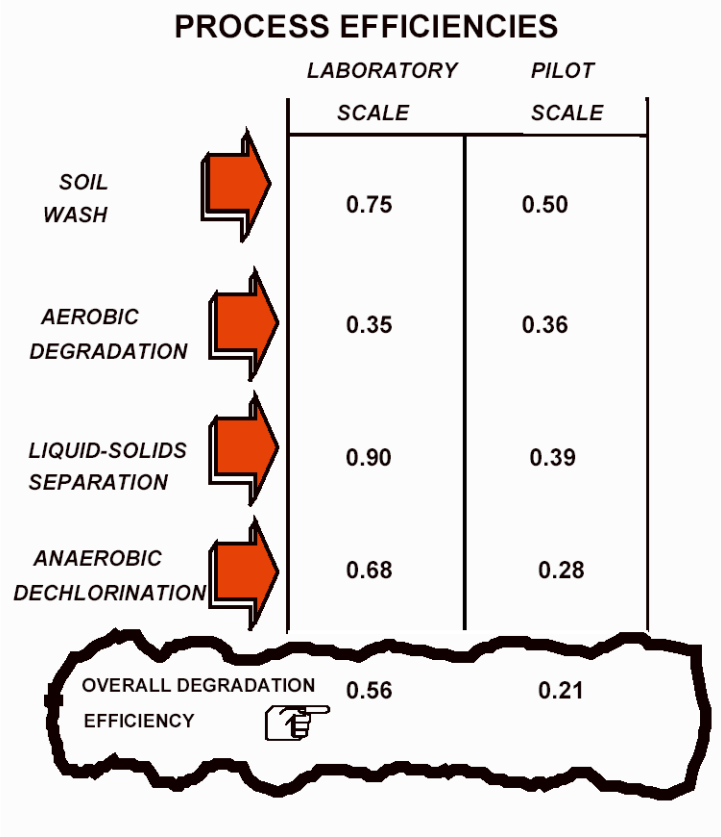

Figure 4-1
Comparison of Processing Step and Overall Process Efficiencies for Laboratory and Pilot Scale Operations of the In Situ Soil Irrigation Process for Biotransformation of PCBs in Soils

It is apparent that some improved efficiency may result from closer attention to the nuances of the various unit operations. The foaming problem during the second aerobic degradation was a likely cause of inefficient PCB degradation and was related directly to operation of the cycle. The soil washing regime appeared to suffer from the increase in scale from laboratory to pilot scale. Unknown variables at increased scale, such as channeling, may take a toll on efficiency of soil washing. The relatively low efficiency of soil washing at pilot scale seriously limited the success of the overall process.

The use of the recombinant bacteria as the FAVs was a novel and valuable research and development venture. The utility of recombinant microorganisms in bioremediation remains a challenging issue. The laboratory and pilot scale tests provided much opportunity for practical experience in the application of recombinant organisms and the challenges in containment of recombinants. The complexity of containment and monitoring for microbial releases during process cycles will likely increase as scale is increased.

The FAVs necessitated the use of the surfactant, POL, for soil washing and the use of a bioreactor for aerobic degradation as opposed to in situ application directly to soil. The FAVs were as efficient at PCB degradation during the first aerobic cycle at pilot as at laboratory scale.

Complete POL metabolism by the FAVs has great potential to improve the subsequent anaerobic dechlorination and may boost the rate and extent of aerobic PCB degradation. Apparently POL degradation did not proceed at as fast a rate at pilot as at laboratory scale. Although a similar amount of POL was metabolized during the pilot scale second aerobic degradation cycle as during the first cycle, PCB degradation suffered. Such measures as nutrient supplementation or a second inoculum of the FAVs may stimulate POL metabolism, but increase cost and complexity of operation. The antagonism between foam control and aeration to keep the DO reading above 3.0 mg/L probably played a role in the diminished PCB aerobic degradation outcomes at pilot scale. It is unlikely that additional means to aerate can be implemented due to the potential for foam.

Residual POL likely reduced the efficiency of the pilot scale anaerobic dechlorination in comparison to the laboratory results. The relatively slow rate of dechlorination will be inherent to any processing scheme. It was accepted that the 8-week incubation was a rather minimal incubation period to provide. The 8-week interval was selected based on the time course of laboratory tests and appeared to be a workable time period. There was no change in PCBs during the second anaerobic dechlorination at pilot scale in 8 weeks. At the end of processing, it was found that the necessity of diatomaceous earth in providing a simulated sediment for deposition of PCBs resulted in significant residual PCBs on equipment. This has potential for confounding a materials balance and for making additional waste treatment problems if not properly controlled at increased scale.

Liquid-solids separations were less efficient at pilot as opposed to laboratory scale. This was due in part to POL concentrations above the CMC during the separation steps. PCBs were apparently contained in micelles. Diatomaceous earth was abrasive and damaging to the pumps and tubing. The equipment configuration proposed for moving solids proved to be somewhat complicated. It was realized at the time of the second liquid-solids separation that operations will benefit from not moving the diatomaceous earth at all.

It was anticipated that a demonstration of the process would take place on the Moccasin Bend Substation. Design plans were drawn for this deployment (data not included). Each capacitor bank would have provided 7774 kg PCB-contaminated interface for the demonstration. At the same time a portable version of the processing units to be skid mounted and used at substations as needed was conceptualized (data not included). A field demonstration did not come to fruition. The capacitor banks were excavated, the PCB-contaminated soil was sent to landfill, and the site was restored to full use. Lessons learned during the pilot scale test created awareness that a number of improvements to streamline the process, decrease equipment needs, and improve unit operation efficiencies are a necessity before venturing to field demonstration.

5
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