Emerging Technologies for the In Situ Remediation of PCB-Contaminated Soils and Sediments: Bioremediation and Nanoscale Zero-Valent Iron

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This report reviews emerging technologies for the in situ remediation of PCB-contaminated sediments and soils to assess their viability for future employment. The target audience is federal and state regulators, planners, and managers responsible for cleaning up soils and sediments contaminated with PCBs. The report is available on the Internet at www.clu-in.org/ studentpapers/.

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I. Purpose

Persistent organic pollutants foul countless aquatic ecosystems worldwide. The remediation of these contaminants is essential to promote public health, environmental quality, and the economy. Polychlorinated biphenyls (PCBs) reside in river sediments for extended durations and bioaccumulate in the food chain through predation (Bedard, 2003). Traditional remediation practices for these contaminants have serious limitations and high costs. The mission of this document is to review emerging technologies for in situ remediation of PCB-contaminated sediments and soils and to assess their viability for future employment. Emphasis is placed on bioremediation and the use of nano-sized zero-valent iron for reductive dechlorination.

II. Characterization of the Problem

PCBs are synthetic aromatic compounds notorious for their recalcitrance and potential toxicity. PCBs comprise two benzene rings connected at the C-1 carbon (Wiegel and Wu, 2000). Each benzene ring can have up to 5 chlorine substituents in the *ortho*, *meta*, or *para* positions (See

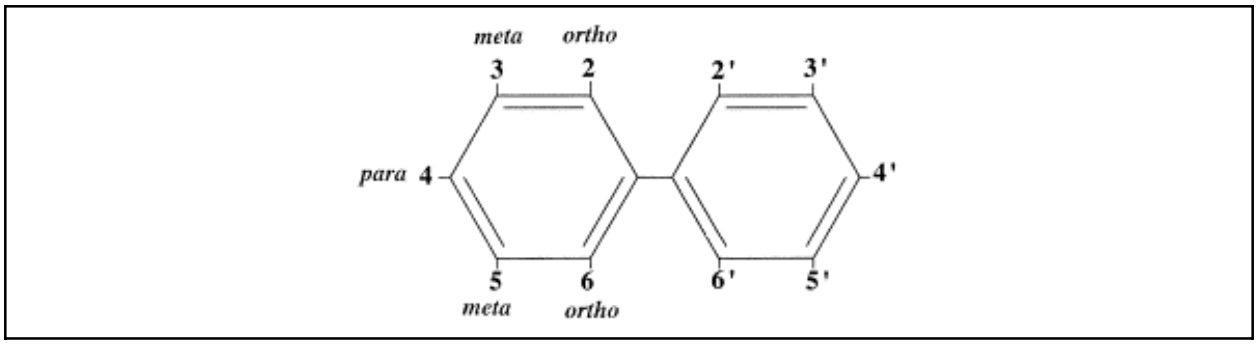

Figure 1. Biphenyl Molecule
(Wiegel and Wu, 2000)

figure 1) (Wiegel and Wu, 2000). PCBs thus have 209 distinct structural arrangements differing in chlorine number and position (Bedard, 2003). Each species is known as a congener and exhibits unique chemical properties (Bedard and May, 1996). In the United States, PCBs were sold commercially as mixtures, most commonly under the trade name Aroclor (Wiegel and Wu, 2000).

American industries manufactured PCBs from 1929 to 1978 primarily for use in electrical transformers and capacitors (Bedard, 2003). PCBs are wonderful insulators characterized by their stability, incombustability, and low volatility (Rodrigues et al., 2000). PCB production in the United States peaked in 1975, as their indestructability made them suitable for a myriad of industrial purposes (Abraham et al., 2002). The widespread use of PCBs inevitably resulted in their deliberate and unintentional discharges into the environment. One-third of all U.S.-produced PCBs currently reside in the natural environment (Wiegel and Wu, 2000).

Once in aquatic or terrestrial systems, PCBs sorb to abiotic or biotic particles due to their hydrophobicity (Mondello, 2002). Heavily chlorinated congeners are the most water insoluble (Mondello, 2002). Of the hundreds of millions of pounds of PCBs released into the environment, most are bound to aquatic sediments (Bedard, 2003). PCBs are recalcitrant to biological degradation because they are so highly oxidized (Mondello, 2002). Furthermore, strongly sorbed PCB molecules are not available to microorganisms capable of PCB degradation. Deposition of clean sediments slowly buries PCB-contaminated particles, reducing the risk of human exposure; however, elevated flows can resuspend contaminated sediments, making PCBs available to aquatic organisms once again (QEA, 1999). The slow desorption of PCBs also pollutes the water column, making the natural recovery of contaminated sediments an ineffective remediation mechanism. PCBs were banned in the United States in 1978 due to growing concern about their toxicity and environmental longevity (Wiegel and Wu, 2000).

III. Public Health Implications

PCBs pose a very real human health threat through numerous exposure pathways. Most alarming is the tendency of PCBs to bioaccumulate, or to increase in concentration while ascending the food chain. PCB concentrations in fish and aquatic mammals can be thousands of times higher than levels in the surrounding waters (Rahuman et al., 2000). Contaminated fish consumption is a major route of PCB bioaccumulation in humans (Johnson et al., 2000). Other exposure avenues are usage of old electrical appliances and inhalation of volatilized PCBs near contaminated sites (Rahuman et al., 2000). Laboratory animals dosed with PCBs developed numerous health problems. Among the adverse health effects were liver damage, skin irritation (acne), reproductive dysfunction, and cancer (Rahuman et al., 2000). Humans exposed to PCBs have an increased risk of developing cancers like non-Hodgkins lymphoma (Johnson et al., 2000). Research also has shown that PCBs can cause severe neurological problems in children, including impairment of cognitive and motor abilities (Faroon et al., 2001). Lipophilic PCBs can be transmitted from mother to child during breast feeding (Faroon et al., 2001).

PCBs are considered most dangerous in their potential for a “dioxin-like toxicity” (Baars et al., 2004). Dioxins are organic aromatic compounds released by industrial processes, seismic emissions, or waste incineration emissions (Baars et al., 2004). They can be chlorinated and are regarded as much more toxic than PCBs. Dioxins cause immunological and reproductive dysfunction and inhibit neurologic growth and development (Baars et al., 2004). The U.S. Environmental Protection Agency regulates dioxins as probable carcinogens, and 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin is considered the most toxic synthetic chemical ever produced (Gruden et al., 2003; Halden and Dwyer, 1997). Dioxin-like PCB congeners contain two chlorines in the *para* position, at least two chlorines in the *meta* position, and at most one chlorine in the *ortho* position (Bedard, 2003). This arrangement allows the PCB molecule to rotate and assume a coplanar orientation, causing the dioxin-like behavior (Baars et al., 2004). While dioxin-like PCBs are more carcinogenic, non-coplanar congeners are more disruptive of

cognitive function (Faroon et al., 2001). To protect public health, all congeners of PCBs must be completely removed from polluted sites available to human exposure.

IV. Traditional Remediation Technologies

Incineration and landfilling are two traditional methods for remediation of PCB-contaminated soils and sediments. High temperature incineration is most commonly used for complete destruction of PCBs (Rahuman et al., 2000). Specialized incinerators burn PCB-contaminated soils or sediments at temperatures up to 1200°C and are required to achieve removal efficiencies of 99.999 percent (U.S. EPA, 1997). There is much public opposition to hazardous waste incineration for fear of exposure to toxic emissions. Furthermore, incineration is very expensive, costing up to $2,300 per ton for a fixed PCB incinerator (U.S. EPA, 1997).

Sequestering liquid PCBs or contaminated soils and sediments in a hazardous waste landfill is another form of disposal (U.S. EPA, 1997). The main associated danger is that the PCBs can volatilize and escape the landfill through surrounding air channels (Rahuman et al., 2000). The failure of leachate collection systems also could result in PCB groundwater infiltration. Landfilling is merely a containment mechanism that does not eliminate the possibility of environmental contamination. The crippling limitation of landfilling and incineration is that they can only be applied ex situ. As a result, dredging of river sediments and soil excavation are necessary precursors to PCB destruction. Aquatic PCB contamination can be temporarily worsened by the dredging process.

Dredging stirs up a fraction of the PCBs formerly tied to sediments, resuspending them in the water (Voie et al., 2002). Dredging also removes organic fine grained sediments, leaving behind coarse inorganics with a lower affinity for PCB binding. As a result, PCBs become temporarily more concentrated in the water column, and consequentially more available for bioaccumulation in aquatic wildlife (Voie et al., 2002). Like incineration, dredging is an expensive procedure. The proposed Superfund dredging of contaminated sediments in the Hudson River will cost upwards of half a billion dollars (U.S. EPA, 2003). Although dredging is proven to be effective in the long run, a more efficient in situ strategy would facilitate the remediation of contaminated soils and sediments.

V. Anaerobic Reductive Dechlorination

Background

In the 1980s, researchers noted discrepancies between commercial Aroclor mixtures and PCBs found in contaminated sediments. The congener distribution of sediment PCBs had a greater proportion of lightly chlorinated species (Wiegel and Wu, 2000). The apparent dechlorination processes occurring naturally in contaminated sediments stimulated extensive laboratory work, as it was once thought that chlorinated synthetic compounds were completely resistant to microbial breakdown (Mondello, 2002). Research soon conclusively demonstrated that anaerobic

organisms were responsible for the PCB dechlorination in aquatic sediments (Bedard, 2003). Anaerobic bacteria replace chlorine substituents with the electron-donating hydrogen (from H_2) on the PCB molecule (Wiegel and Wu, 2000).

In general, microbial reductive dechlorination of PCBs removes *meta* and *para* chlorines from highly chlorinated congeners, resulting in predominately *ortho* substituted mono- through tetrachlorobiphenyls (Wiegel and Wu, 2000). There are eight major dechlorination pathways known to date, each differing in congener and position reactivity (Bedard, 2003). Figure 2 reviews the known microbial dechlorination processes. The most extensive dechlorination occurs when process M works in combination with process Q. This activity, known as C

Dechlorination Pathway	Chlorines Removed
M	Flanked and unflanked *meta*
Q	Flanked and unflanked *para*, *meta* of 2,3-
H’	Flanked *para*, *meta* of 2,3- and 2,3,4-
H	Flanked *para*, doubly flanked *meta*
P	Flanked *para*
N	Flanked *meta*
LP	Flanked and unflanked *para*
T	Flanked *meta* of 2,3,4,5- in hepta- and octachlorobiphenyls

Figure 2. Microbial Dechlorination Pathways
“Flanked” signifies an adjacent chlorine
(Wiegel and Wu, 2000)

dechlorination, voraciously attacks *meta* and *para* chlorines, resulting in exclusively *ortho* substituted congeners (Zwiernik et al., 1998). This process is advantageous because lightly chlorinated *ortho* substituted species are non-dioxin-like and do not readily bioaccumulate. Unfortunately, only Hudson River sediments have expressed process C dechlorination in situ (Zwiernik et al., 1998). While no defined *ortho* dechlorination pathways exist, enriched cultures derived from Baltimore Harbor estuarine sediments exhibit significant *ortho* dechlorination (Berkaw et al., 1996).

Analysis of Dechlorinating Populations

Characterization of the anaerobic organisms responsible for PCB dechlorination is paramount to the development of a remedial scheme. PCB reduction is known to occur as a cometabolic process and is believed to occur as a product of dehalorespiration (Abraham et al., 2002). Methanogens and sulfate reducers are largely responsible for dechlorination pathways H, M, and Q (Bedard, 2003). Spore-forming sulfate reducers are the most important, essential for the *para* dechlorination in process Q (Zwiernik et al., 1998). Furthermore, Fava reports that spore-forming sulfate reducers are necessary for M dechlorination (Fava et al., 2003a). M dechlorination does not proceed in the presence of molybdate, an inhibitor of sulfate-reducing bacteria (Fava et al., 2003a). Sulfate-reducing bacteria are thus responsible for C dechlorination, the most prolific dechlorination process found in contaminated sediments.

Dehalorespiration refers to microbial use of halogenated compounds as terminal electron acceptors for energy synthesis (Rosenthal et al., 2004). The identification of a PCB-respiring organism would be invaluable to the advancement of PCB bioremediation. Such a microbe could use PCB for growth, thus having a distinct advantage in PCB-contaminated sediments. Kim and Rhee demonstrated that such dechlorinating organisms exist in sediments independent of sulfate reducers or methanogens (Kim and Rhee, 1997). The study showed that components of an anaerobic consortium required Aroclor 1248 for growth, disappearing below a threshold concentration (Kim and Rhee, 1997). The populations of sulfate reducers and methanogens were sustained in the absence of the Aroclor (Kim and Rhee, 1997).

The Identification of Two Dechlorinating Organisms

Organism *o*-17

In the past few years, two different individual species have been identified that catalyze the reductive dechlorination of PCBs. The first such microbe is bacterium *o*-17, which depends on the presence of 2,3,5,6-tetrachlorobiphenyl for growth (Cutter et al., 2001). Cutter derived *o*-17 from the *ortho* dechlorinating consortium of Baltimore Harbor. In sediment-free media, *o*-17 reduces congeners 2,3,5,6- and 2,3,5-chlorobiphenyl to 3,5-chlorobiphenyl. Acetate is a potential electron donor for the process, as *o*-17 requires acetate for dechlorination (Cutter et al., 2001). Addition of hydrogen to the medium inhibited dechlorination, suggesting that hydrogen is not the main electron donor. Yet hydrogen produced from the oxidation of acetate might serve as the donor, so the oxidation-reduction mechanism remains unclear (Cutter et al., 2001). Attempts to isolate *o*-17 as a pure culture have been unsuccessful (Cutter et al., 2001).

As the *ortho* position is notoriously resistant to reductive dechlorination, the discovery of *o*-17 constitutes a major breakthrough. Phylogenetically, *o*-17 is most similar to *Dehalococcoides ethanogenes*, a hydrogenotrophic organism that respires through the dechlorination of tetra-chloroethene (Cutter et al., 2001). The two organisms and the chlorobenzene dechlorinating strain *Dehalococcoides* CDB1 belong to a phylogenetic branch closely related to the green non-

sulfur bacteria (Cutter et al., 2001). The fact that *o*-17 strongly resembles the only known organisms capable of dehalorespiration is very encouraging (Abraham et al., 2002). Unfortunately, the estuarine origin of *o*-17 limits its compatibility with the environmental conditions of soils and sediments. Such site-specific dechlorination activity is unlikely to evolve into a ubiquitous remedial solution.

Organism DF-1

Wu discovered another organism with growth linked to the reductive dechlorination of PCBs (Wu et al., 2002). Bacterium DF-1 dechlorinates doubly flanked chlorines on the biphenyl molecule (Wu et al., 2002). DF-1 can remove *meta* chlorines from 2,3,4-chlorobiphenyl and 2,3,4,6-chlorobiphenyl, and *para* chlorines from 3,4,5-chlorobiphenyl and 2,3,4,5-chlorobiphenyl (Wu et al., 2002). DF-1 was identified as the responsible dechlorinator from a culture containing mainly sulfate reducers; thus, the extensive dechlorination capacity of sulfate-reducing consortia might be attributable to specific dechlorinating bacteria, such as DF-1. The bacterium most closely resembles *o*-17 (89 percent rDNA sequence similarity), further advancing the thought that a class of PCB dechlorinators exists in the natural environment (Wu et al., 2002). DF-1, like *o*-17, has yet to be isolated as a pure culture (Wu et al., 2002).

Biostimulation

The two major bioremedial actions are biostimulation and bioaugmentation. Biostimulation involves the addition of a primer to galvanize targeted dechlorinating populations. A very successful laboratory study stimulated process C dechlorination through the addition of ferrous sulfate to PCB-contaminated soils (Zwiernik et al., 1998). $FeSO_4$ amendments saturate aqueous systems with free sulfate, which is consumed by sulfate reducers. Bioenergetics favor the sulfate reducers over the methanogens, and sulfate-reducing populations grow rapidly, while methanogenic growth is inhibited (Zwiernik et al., 1998). PCB dechlorination is initially inhibited as sulfate becomes the primary electron acceptor for microbial respiration. Once sulfate is depleted, PCB dechlorination resumes as the sulfate reducers attack *para* chlorines, supplementing the more common *meta* dechlorination observed in the unamended controls (Zwiernik et al., 1998). The result is nearly complete C dechlorination of Aroclor 1242, resulting in the accumulation of the *ortho* substituted congeners 2-chlorobiphenyl and 2,2'/2,6-chloro-biphenyl (Zwiernik et al., 1998). This process has great potential for in situ application, as it was postulated that priming one ton of sediment requires only 10.6 pound of ferrous sulfate, a cheap and environmentally benign product (Bedard, 2003).

Another way to “prime” anaerobic sediments for PCB dechlorination is through addition of bromobiphenyls. A field study in Woods Pond of the Housatonic River demonstrated that spiking sediments with 2,6-bromobiphenyl stimulated reductive dechlorination (Bedard, 2003). One 350 µM pulse of the bromobiphenyl activated native PCB dechlorinators, resulting in a 74 percent decrease in PCBs with six or more chlorines in just one year (Bedard, 2003). The bromobiphenyl primer resulted in N dechlorination, yielding mainly *ortho* and *para* substituted tetrachlorobi-

phenyls (Bedard, 2003). Inoculation of sediments with N dechlorination products can prime sediments for LP dechlorination, resulting in mostly *ortho* substituted dichlorobiphenyls (Bedard, 2003). The downside of using halogenated aromatics as primers is that they are recalcitrant to degradation (Abraham et al., 2002). The practice of adding more of a contaminant to a site to stimulate microbial action is unacceptable to regulators. Halobenzoates are easily mineralized and are thus more suitable as dechlorination primers. Chlorobenzoates result from the aerobic oxidation of lightly chlorinated PCBs. Interestingly, they successfully stimulate dechlorination only in sediments other than their sediments of origin (Abraham et al., 2002). Priming is necessary to incite and expedite the reductive dechlorination of PCBs.

Bioaugmentation

Bioaugmentation is the process of enriching a contaminated site with organisms capable of degrading a targeted compound. Attempts to augment PCB-dechlorinating cultures in Housatonic River sediments have been unsuccessful (Bedard, 2003). These studies inoculated the sediments with enriched cultures indigenous to the Housatonic River (Bedard, 2003). Augmentation with cultures from different PCB-contaminated sediments might have worked better, just as chloro-benzoates only prime dechlorination in non-native sediments. One successful augmentation study used a granular anaerobic methanogenic microbial consortium (Natarajan et al., 1996). The granules were produced by an upflow anaerobic sludge-blanket reactor with a continuous supply of carbon and electron sources (Natarajan et al., 1996). In the laboratory, the methanogenic granules completely dechlorinated 2,3,4,5,6-pentachlorobiphenyl to biphenyl. Dechlorination to biphenyl was an unprecedented accomplishment. The granules removed chlorines from all feasible positions in the presence of glucose and methanol (Natarajan et al., 1996).

In a subsequent study, the granular consortium was added to PCB-contaminated sediments from the River Raisin (Natarajan et al., 1997). Sediment amended with the granules experienced a significant reduction in tri- through heptachlorobiphenyls (Natarajan et al., 1997). The primary dechlorination products of the original Aroclor 1242 and 1248 mixtures were *ortho* substituted mono- and dichlorobiphenyls (Natarajan et al., 1997). Control sediments without the inoculum underwent very slight dechlorination, illustrating the success of the bioaugmentation. Also encouraging was that the granules dechlorinated at a wide range of ambient temperatures (Natarajan et al., 1997). Potentially problematic is that the bench-scale experiments required a volume of granules equal to 10 percent of the treated sediment volume (Bedard, 2003). Questions remain as to how the efficiency of the consortium translates to full-scale field projects. The last few years have shown no advances in the granular technology, which suggests that it is not as promising as once imagined.

Technology Assessment

There is great potential for in situ remediation of PCB-contaminated sites using anaerobic reductive dechlorination. Dechlorination pathways have been identified along with two organisms that catalyze the reductive dechlorination of PCBs. DF-1 and *o*-17 are very similar to

each other and to *Dehalococcoides ethenogenes*, a bacteria known to halorespire on tetrachloroethene (Wu et al., 2002). Even more intriguing is a recent study reporting that *Dehalococcoides (Dhc) ethenogenes* strain 195 dechlorinates 2,3,4,5,6-pentachlorobiphenyl to 2,3,4,6- and/or 2,3,5,6-tetrachlorobiphenyl and 2,4,6-trichlorobiphenyl (Fennell et al., 2004). Researchers did not test *Dhc* 195 for growth on PCB, but the strain was shown to use chlorinated benzenes as electron acceptors (Fennell et al., 2004). This report supports the idea that certain *Dhc* species are involved in the natural reductive dechlorination of PCBs. Research must determine if *Dhc* strains are able to use PCB for growth.

At present, anaerobic reductive dechlorination is not a viable stand-alone alternative to dredging/excavation and burning. More field studies must be conducted to test methods of bioaugmentation and biostimulation. The behavior of PCB-dechlorinating enrichment cultures has not been evaluated in situ. Methods of priming dechlorination are established, but their field applicability is unknown. Pure culture isolation of a PCB dechlorinator is essential in developing a better understanding of the relevant microbial processes (Wiegel and Wu, 2000). As described below, a recent attempt at field-scale remediation of PCBs was largely unsuccessful, but it is useful for illustrating the remaining barriers to biotic dechlorination of PCBs.

Analysis of a Summer 2003 Field Study

In the summer of 2003, the Army Corps of Engineers sponsored a field-scale bioremediation test on PCB-contaminated soils in Mississippi (Tiedje, 2004). The project sought to mineralize a mixture of Aroclors 1242/1248 through a sequential anaerobic/aerobic treatment (Tiedje, 2004). Researchers from Michigan State devised the remediation scheme with the idea that anaerobic reductive dechlorination would reduce chlorination to levels low enough for aerobic oxidation to cleave the biphenyl molecule. Unfortunately, attempts to stimulate reductive dechlorination were unsuccessful (Tiedje, 2004). Researchers applied PCB-contaminated sediment and a carbon source to the flooded soil in an effort to trigger dechlorinators already present in the soil. After six months, no substantial dechlorination was observed, and the Corps terminated the project (Tiedje, 2004).

The project in Mississippi highlights the shortcomings of anaerobic reductive dechlorination as a remedial process for PCB-contaminated soils and sediments. Significant dechlorination can take several years under optimal environmental conditions (Tiedje, 2004). The six-month time limit was highly unreasonable. Aside from time constraints, the limited bioavailability of PCBs severely inhibits reductive dechlorination. PCBs are often tightly bound to soil and sediment particles, rendering them resistant to the enzymes of dechlorinators (Richardson, 2004). Furthermore, it is very difficult to establish and stimulate PCB-dechlorinating organisms in remediation sites. Threshold PCB concentrations exist for the successful maintenance of dechlorinating cultures that might not be abundant in the first place (Cho et al., 2003). The interactions of the mechanisms involved must be studied further, along with the properties of the PCB dechlorinators themselves.

VI. Aerobic Biodegradation

Background

Preliminary laboratory research on aerobic PCB biodegradation was discouraging. Researchers tried to identify organisms capable of utilizing highly chlorinated PCBs as carbon sources for growth (Mondello, 2002). In 1973, Ahmed and Focht reported that *Achromobacter* degrades a few lightly chlorinated PCBs as a cometabolic function of biphenyl oxidation (Mondello, 2002). It is now well known that PCBs are broken down by the catabolic "biphenyl pathway" (or *bph* pathway) (Sylvestre, 2004). The *bph* pathway is a four-step enzymatic process that turns biphenyl into benzoic acid and 2-hydroxy-penta-2,4-dienoic acid (Bedard, 2003) (See figure 3 for *bph* pathway schematic). The pentanoic acid product is effectively converted to acetyl-CoA and used in the tricarboxylic acid cycle (Bedard, 2003). In general, PCBs with at most three chlorines are susceptible to degradation via the *bph* pathway (Mondello, 2002).

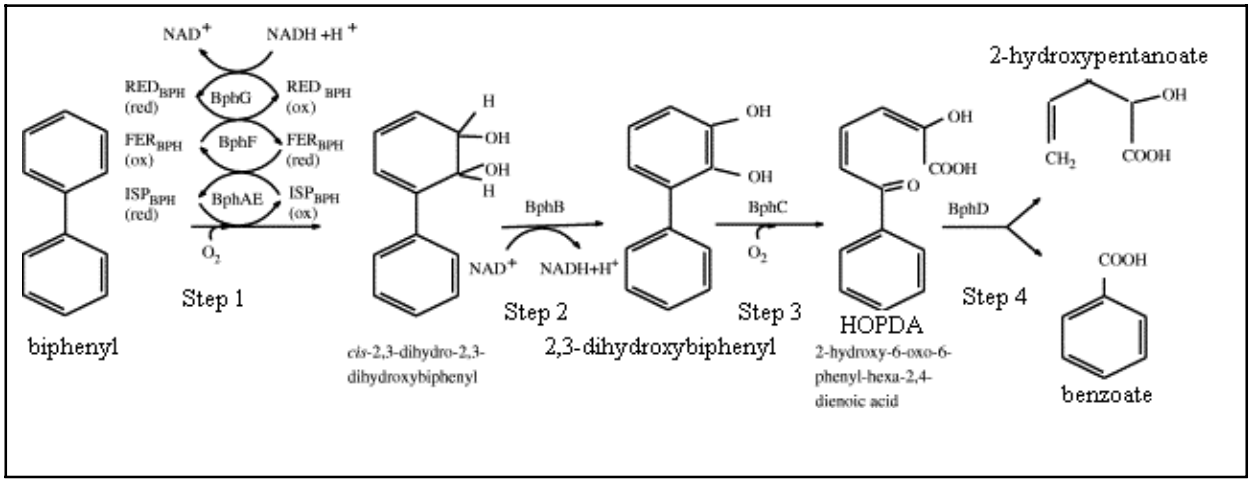

Figure 3. Biphenyl (*bph)* Pathway
(Sylvestre, 2004)

Analysis of PCB-Degrading Populations and Mechanisms

A broad range of gram-negative and gram-positive aerobic bacteria encoding the biphenyl pathway are capable of cometabolically degrading PCBs. The large majority use biphenyl dioxygenase to attack 2,3- carbons and form 2,3-dihydrodiol (Mondello, 2002). Other dioxygenases subsequently produce 2,3-dihydroxychlorobiphenyl, which is cleaved at the *meta* position to yield chlorinated 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA) (Sylvestre, 2004). This stage can be a "bottleneck" for certain PCB congeners because 3- and 4-chloroHOPDA competitively inhibit HOPDA hydrolase (Bedard, 2003; Sylvestre, 2004). If uninhibited, the hydrolase splits HOPDA into chlorobenzoic acid and a five-carbon compound (Mondello, 2002).

Bukholderia cepacia LB400 and *Ralstonia eutropha* H850 are distinguished by their broad congener specificities, and thus have been the subjects of extensive research. LB400 is the most capable PCB degrader and is not pathenogenic like its relative, *Burkholderia pseudomallei* (Kenyon College, 2004). LB400 and H850 are unique because they can attack PCBs without unchlorinated 2,3- positions (Mondello, 2002). The biphenyl oxygenase of LB400 and H850 has an acute affinity for 2- and 2,4-chlorophenyl rings at the *ortho* position. As a result, they can use oxygenolytic dehalogenation to spontaneously produce dihydroxybiphenyl (Bedard, 2003). A number of competent gram-positive PCB degraders belong to the *Rhodococcus* genus (Mondello, 2002).

Genetic Engineering for PCB Mineralization: Strain RHA(pRHD34)

The mineralization of PCB by the biphenyl pathway is extremely rare. Most of the time, the enzymes degrade the ring with fewer chlorines while releasing the second ring as a chlorobenzoic acid (CBA) (Abraham et al., 2002). Natural PCB degraders are unable to catalyze the degradation of chlorobenzoates, leading to a buildup of the metabolite (Rodrigues et al., 2000). This is problematic because CBAs can be toxic and inhibitory to PCB degraders (Rodrigues et al., 2000). As a result, genetic engineering has become a necessary tactic to produce organisms with the *bph* pathway and a CBA degradation pathway. An organism capable of completely mineralizing a wide range of congeners would advance bioremediation as a viable alternative for PCB-contaminated sites.

Aerobic degradation of PCBs is severely limited by the inability of naturally occurring organisms to grow on and fully metabolize the PCB molecule (Rodrigues et al., 2000). Efficient PCB destruction by the *bph* pathway is dependent upon the availability of biphenyl as a co-substrate (Manzano et al., 2003). A major development was the construction of a recombinant *Rhodococcus* RHA1 strain capable of growing on PCB in non-sterile soil media (Rodrigues et al., 2000). RHA1 degrades a wide range of PCBs and co-contaminants, like benzene (Bedard, 2003). Naturally occurring RHA1 does not use PCB as a carbon source, and cannot degrade the chlorobenzoic acids that accumulate as a product of PCB cometabolism (Rodrigues et al., 2000). Researchers first identified the *fcb* operon as the genes encoding for the hydrolytic dechlorination of 4-CBA (Rodrigues et al., 2000). The operon was cloned into the RHA1 strain to supplement the already present *bph* pathway. The resulting modified organism, RHA(pRHD34), was able to grow on and degrade 4-chlorobiphenyl without subsequent accumulation of 4-CBA (Rodrigues et al., 2000).

Wild-type RHA1 converts approximately 60 percent of process M dechlorination products to corresponding CBAs (Rodrigues et al., 2000). The recombinant strain breaks down PCBs with similar efficiency and completely degrades 4-CBA. RHA(pRHD34) also reduces *meta* cleavage products that can inhibit enzymatic function (Rodrigues et al., 2000). The *fcb* operon was stable in the non-sterile media for 60 days, which should be more then enough time for an aerobic field remediation project (Rodrigues et al., 2000).

RHA(pRHD34) shows great promise for remediation of PCBs but has many limitations. For one, growth on 4-chlorobiphenyl is only feasible through partial induction of the *bph* pathway. Full expression of the *bph* pathway produces 4-CBA faster than the *fcb* pathway can break it down (Bedard, 2003). The result is accumulation of 4-chloroHOPDA that inhibits growth of the recombinant strain (Rodrigues et al., 2000). Partial induction of *bph* ameliorates this problem but limits the range of PCBs degraded by the recombinant strain (Bedard, 2003). It is essential, therefore, to engineer organisms with the *fcb* operon that still allow full expression of *bph* (Bedard, 2003).

Problems in the Pathway

A key bottleneck has been identified in the third step of the *bph* pathway (Dai et al., 2002). The third-step enzyme responsible for aromatic ring cleavage is 2,3-dihydroxybiphenyl 1,2-dioxygenase (DHBD) (See figure 4). Dai et al. conclusively demonstrated that *ortho* chlorinated

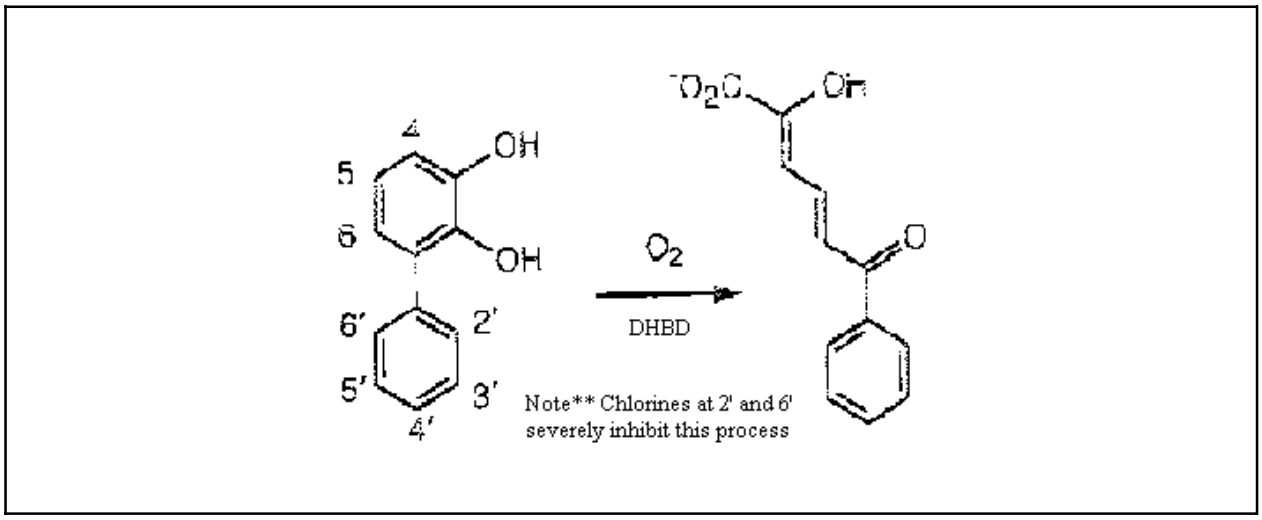

Figure 4. DHBD Cleavage
(Dai et al., 2002)

2,3-dihydroxybiphenyls bind to and inhibit DHBD, promoting the enzyme's suicide inactivation (Dai et al., 2002). This is problematic because chlorinated 2,3-dihydroxybiphenyls are PCB degradation products of the *bph* pathway. 2',6'-Dichloro-2,3-dihydroxybiphenyl has the greatest affinity towards DHBD and causes suicide inactivation through the oxidation of active site Fe(II) (Dai et al., 2002). As a result, ring cleavage of many *ortho* substituted congeners is extremely difficult. Even the voracious PCB degrader *Burkholderia* LB400 is severely limited in its ability to degrade doubly *ortho* substituted congeners. LB400 transforms less than 5 percent of 2,6-dichlorbiphenyl to its analogous chlorobenzoic acid (Dai et al., 2002). Also discouraging is a new study proving that dihydrodiols are significantly toxic to aerobic bacteria (Cámara et al., 2004). As dihydrodiols are the products of the first step in the biphenyl pathway, there is no feasible way to

circumvent this problem. Finding a way to mitigate this toxicity might enable more rapid and complete PCB destruction (Cámara et al., 2004).

A Superior Recombinant Strain: LB400(pR041)

The potential for aerobic bioremediation of PCBs is greatly increased by the recent development of a superior LB400 strain (Tiedje, 2001). Researchers at Michigan State have successfully engineered LB400(pR041) to carry the *ohbRABC* operon for degradation of *ortho* substituted chlorobenzoates (Denef et al., 2003). The *ohb* genes were derived from *Pseudomonas aeruginosa* 142, which can degrade 2-CBA and 2,4-diCBA (Tsoi et al., 1999). LB400(pR041) effectively grows on and mineralizes many *ortho* substituted PCBs (Tiedje, 2001). As a result, the strain can be used to completely break down the majority of congeners evolved by anaerobic reductive dechlorination (Tiedje, 2004). The mineralizing activity of LB400(pRO41) also prevents the buildup of potentially toxic dechlorinated metabolites (Tiedje, 2001). The genome of LB400 has been successfully closed by the Michigan State team, providing for a further understanding of its PCB metabolism (Tiedje, 2004). The recombined LB400 strain is very stable in non-sterile soil, and is easily the most promising organism for use in the aerobic stage of the anaerobic/aerobic bioremediation sequence (Tiedje, 2004).

Remaining Barriers and Possible Remedies

The shortcomings of LB400(pRO41) highlight the current limitations of aerobic bioremediation of PCBs. Most notably, LB400(pRO41) is unable to degrade doubly *ortho* substituted congeners (Tiedje, 2004). PCBs with chlorines in the 2,2'- or 2,6- positions are recalcitrant to the recombined strain, remaining extremely problematic (Tiedje, 2004). The impaired degradation of these congeners is potentially caused by the aforementioned suicide inactivation of DHBD by *ortho* substituted dihydroxybiphenyls. To improve aerobic degradation of *ortho* substituted congeners like 2,6-dichlorobiphenyl, Dai et. al propose that directed evolution of DHBD could enable it to more effectively cleave 2',6'-dichloro-dihydroxybiphenyl. Another potential solution is to lower the binding affinity of DHBD to 2',6'-dichloro-dihydroxybiphenyl (Dai et al., 2002). LB400(pRO41) is also susceptible to HOPDA inhibition, which prevents the efficient degradation of many congeners (Tiedje, 2004; Bedard, 2003). The degradation of PCBs with chlorines on both biphenyls is especially sensitive to HOPDA inhibition as the metabolic formation of 3- and 4-chloroHOPDAs is more likely (Sylvestre, 2004).

An encouraging study published in 2004 reports that significant differences exist between the HOPDA hydrolases of homologous organisms LB400 and *R. globerulus* P6 (Sylvestre, 2004). The two hydrolases have varying HOPDA affinities and thus are inhibited by different chloroHOPDAs (Sylvestre, 2004). An in-depth understanding of the mechanisms involved may assist the engineering of a more capable HOPDA hydrolase (Sylvestre, 2004). To further increase the scope of PCB congeners degraded aerobically, additional CBA pathways must be identified and recombined into a PCB degrader. 2,4-CBA, 2,5-CBA, and 2,6-CBA would be most advantageous

(Bedard, 2003). Such pathways might enable organisms like LB400 to mineralize the enigmatic doubly *ortho* substituted PCBs.

Revisiting the 1991 GE Hudson River Field Study

The last major field-scale aerobic bioremediation attempt was conducted by General Electric (GE) in 1991. GE drove several caissons into Upper Hudson River sediments in attempt to stimulate in situ aerobic biodegradation (Harkness et al., 1993). Nutrient supplements were provided, as well as hydrogen peroxide as a source of dissolved oxygen (Bedard, 2003). The sediments in the cassions were stirred to establish at least a minimal degree of mixing (Bedard, 2003). The amount of PCB destruction was hard to measure, but there was nowhere near complete conversion of PCBs to corresponding CBAs (Harkness et al., 1993). The major problem was once again the limited bioavailability of the PCBs. The most recalcitrant PCBs were strongly sorbed within the polymeric organic sediment matrix, through which PCBs must diffuse prior to desorption (Harkness et al., 1993). Inoculation of one caisson with H850 proved ineffective, and any PCB degradation was attributed to indigenous populations (Harkness et al., 1993), which was surprising because the H850 was isolated from sediments in the Upper Hudson (Bedard, 2003). As with anaerobic reductive dechlorination, bioaugmentation with indigenous organisms for aerobic bioremediation did not seem to work.

It would be very interesting to conduct a similar test inoculating an aerated, well-mixed caisson with LB400(pRO41) and/or RHA(pRHD34). This approach may have worked well in the Upper Hudson, as a large buildup of 2-CBA was noted in the GE caissons (Harkness et al., 1993). LB400(pR041) carries the *ohb* pathway and thus would have mineralized any evolved 2-CBA (Tsoi et al., 1999). For such a remedial scheme to be successful, PCB bioavailability must be improved. Another difficult task is maintaining sufficient oxygen concentrations for the aerobic organisms (Bedard, 2003). A high degree of mixing is necessary to thoroughly remediate buried contaminated sediments. Such stirring must be controlled to avoid scouring PCBs into the water column. Extensive field-scale research is imperative to elucidate the many factors complicating the in situ aerobic bioremediation of PCB-contaminated sediments.

VII. Reductive Dechlorination by Nanoscale Zero-Valent Iron

Background

Nanotechnology is rapidly expanding the limits of current remediation technologies. Nano-sized particles have diameters between 10^{-9} and 10^{-7} meters and are characterized by crystalline shapes and lattice structures (Masciangioli and Zhang, 2003). Nanoscale zero-valent iron particles have been shown to reduce a wide range of environmental pollutants like halogenated chlorinated solvents (Masciangioli and Zhang, 2003). Nanoscale metals have high surface area-to-volume ratios, high surface energies, and a large fraction of stepped surface (Wang and Zhang, 1997). Such properties combine with a unique structure and zero valency to make nano-sized metals extremely chemically reactive (Masciangioli and Zhang, 2003). Current research is exploring the

ability of these particles to reductively dechlorinate PCBs. Chemical reduction of highly chlorinated PCBs is greatly preferable to chemical oxidation, which can produce toxic dioxin precursors like chlorophenols and chlorocatechols (Jackman et al., 1999).

Demonstrating the Potential of Nanoscale ZVI

Reductive dechlorination of PCBs by nanoscale zero-valent iron (ZVI) was first reported by Zhang and Wang (Wang and Zhang, 1997). Nanoscale ZVI was synthesized through the drop-wise addition of 1.6 M $NaBH_4$ to 1.0 M $FeCl_3 \bullet 6H_20$, which reduces Fe(III) to Fe(0) (Wang and Zhang, 1997). Researchers plated nanoscale zero-valent palladium (Pd) on some of the nano-ZVI to assess its potential as a dechlorination catalyst (Wang and Zhang, 1997). Palladized ZVI was previously shown to completely dechlorinate Aroclors 1254 and 1260 in a short amount of time (Grittini et al., 1995). Nano-Pd/Fe(0), regular nano-Fe(0), and commerical ZVI powder were compared for their abilities to dechlorinate aqueous (5 mg/L PCB) Aroclor 1254 at an ambient temperature in an ethanol/water solution (Wang and Zhang, 1997). Ethanol serves as a solvent for PCBs and the Pd coating. Fe and Pd/Fe were added to an initial concentration of 5 g metal/100 mL solution and left in solution for 17 hours (Wang and Zhang, 1997).

No dechlorination was detected in samples amended with commercial ZVI powder having a specific surface area of 0.9 m^2/g (Wang and Zhang, 1997). Nanoscale ZVI (BET-specific surface area = 33.5 m^2/g) performed better, but still degraded at most only 25 percent of the PCBs initially present. More encouraging was the accumulation of biphenyl in the sample, proving that certain congeners were completely dechlorinated by the ZVI (Wang and Zhang, 1997). The higher specific surface area of the nanoparticles increased contact between the iron and the PCBs, facilitating reduction. The most impressive result was that the zero-valent nanoscale Pd/Fe complex completely dechlorinated Aroclor 1254. After 17 hours, biphenyl was the only detectable dechlorination product (Wang and Zhang, 1997).

Palladium coatings catalyze PCB dechlorination in the presence of a solvent by releasing hydrogen previously absorbed from the surface of the iron (Korte, 2000). Upon release, the hydrogen displaces chloride on the PCB molecule (Korte, 2000). The Pd coating increases ZVI longevity by preventing the formation of iron oxides (Wang and Zhang, 1997). Nanoscale Pd/Fe complexes have immense potential for the total dechlorination of PCBs, but unfortunately the palladium coating adds a substantial production cost (Lowry, 2004). The experiment did not investigate congener-specific dechlorination patterns or the effectiveness of nano-ZVI over an extended period of time.

Analysis of ZVI Positional Preferences

Yak examined the dechlorination of PCBs by ZVI in subcritical water (Yak et al., 1999). Subcritical water, characterized by extremely high temperatures and pressures, acts as a solvent to allow significant dechlorination of Aroclor 1260 by commercial ZVI powder (Yak et al., 1999). The ZVI converted all higher chlorinated PCBs to more lightly chlorinated congeners in a step-

wise fashion. PCBs with lower chlorine contents were more recalcitrant to reduction, but still evolved to biphenyl (Yak et al., 1999). A subsequent study by the same researchers examined the position-specific reductive dechlorination of PCBs in subcritical water (Yak et al., 2000). Interestingly, the patterns were very similar to those of anaerobic microbial dechlorination. The ZVI preferentially dechlorinates *para* chlorines followed by *meta* chlorines. As with microbial processes, *ortho* chlorines are the most recalcitrant to reductive dechlorination by ZVI (Yak et al., 2000). It is hypothesized that the *ortho* substituted PCBs are more resistant because their non-coplanar orientation prevents free spinning along the C-1 carbon. This causes the electron cloud of an *ortho* chlorine to hover over the opposite phenyl ring, effectively preventing reduction (Yak et al., 2000). The results of this study prove that even ZVI has problems dechlorinating *ortho* substituted congeners.

PCB Dechlorination by Micro- and Nanoscale ZVI in Contaminated Sediments

A remarkable study conducted by Dr. Kevin Gardner at the University of New Hampshire demonstrates that ZVI can rapidly and extensively dechlorinate PCBs in contaminated sediments (Gardner, 2004). Gardner injected microscale ZVI into PCB-laden sediments (Fe mass = 3% sediment mass) from New Bedford Harbor and the Housatonic River (Gardner, 2002). The preliminary results were phenomenal. The ZVI removed an estimated 84 percent of PCBs from the Housatonic River sediments in a single day. New Bedford Harbor sediments showed more modest results with an estimated 56 percent removal over the same time period. Substantial biphenyl production was observed, indicating complete dechlorination of PCBs (Gardner et al., 2004). The variance between the two sediments can be attributed to differing PCB availabilites. Housatonic River sediments are loose and sandy and therefore do not strongly sorb to PCBs. New Bedford sediments contain more clay and possess a “slow” desorption fraction. PCBs in the Housatonic are thus more susceptible to attack by ZVI, as it is thought that PCBs must be in the aqueous phase to be reduced (Gardner, 2004). Another interesting result of the experiments was that the ZVI dechlorinated *ortho* chlorines almost as well as *meta* and *para* substituted chlorines (Gardner et al., 2004). The finding of extensive *ortho* dechlorination by ZVI contradicts previous studies, but if valid constitutes a major breakthrough.

The results of the UNH experiment are encouraging, but mysterious. Such efficient reduction by microscale ZVI was unheard of, and even nanoscale ZVI (with a much greater BET-specific surface area) degrades less than 25 percent of PCBs in a water/ethanol solution (Wang and Zhang, 1997). The UNH researchers also performed the same experiment with nanoscale ZVI and achieved very similar results (Gardner, 2004). Confidence in the laboratory data is shaken by the inability of Dr. Gardner to close the PCB mass balances. Biphenyl production was observed, but the amount of biphenyl present did not correspond to the amount of PCB removed (Gardner, 2004). While the PCB removal efficiencies of Dr. Gardner’s tests are superb, his method will not be generally accepted until he is able to account for all of the PCBs initially present in the samples. As a result, Dr. Gardner has gone “back to the drawing board” (Gardner, 2004). Dr. Gardner and New Hampshire researchers are currently working on the reductive dechlorination of

sediment-bound PCBs by zero-valent palladized magnesium in the presence of an ethanol surfactant (Gardner, 2004). Results from these experiments are pending (Gardner, 2004).

Conflicting Research

Recent laboratory work by Dr. Gregory Lowry and fellow Carnegie Mellon researchers casts doubt on the success of Dr. Gardner's sediment experiments. The CMU team developed an "active" sediment cap to degrade or sequester contaminants as they slowly desorb from underlying sediments (Lowry, 2004). The incorporation of ZVI in the cap should dechlorinate desorbing PCBs. To assess this hypothesis, Dr. Lowry tested the aqueous PCB dechlorinating ability of micro- and nanosized ZVI at ambient conditions (Lowry, 2004). Dr. Lowry found that microscale ZVI did not react with PCBs in a 45-day test period (Lowry et al., 2004). Nanosized ZVI dechlorinated PCBs with congener half-lives ranging from 40 days to 77 years (Lowry et al., 2004). No biphenyl production was noted (Lowry et al., 2004). These results vary dramatically from the near-complete dechlorination with microscale iron observed by Dr. Gardner in a single day. Furthermore, Lowry reports that the nano-ZVI exhibited a significant dechlorination preference of *para* and *meta* chlorines over *ortho* chlorines (Lowry et al., 2004). Nanoscale ZVI was not used in the active cap due to the noncompetitive cost of iron at the time (Lowry et al., 2004).

Improving ZVI Longevity

Aside from its high cost, the short reactive life span of nanoscale ZVI impedes its field applicability (Lowry, 2004). For remediation of the strongly sorbed PCBs, ZVI must remain active in sediments and soils for many years. Ideally, an active sediment cap has a design life of hundreds of years (Lowry, 2004). Nanoscale ZVI is so unstable and prone to oxidation that such longevity is not feasible. Coating ZVI with palladium substantially increases the reactive life span as previously described. Current research explores how different methods of Pd incorporation affect ZVI deactivation rates. Traditional construction of the Pd/Fe complex plates Pd on acid-washed base materials (Pd-Fe-A) (Gui and Gillham, 2002). A new alternative method coats palladium on unwashed oxide-covered iron particles (Pd-Fe-U). Researchers assessed the reactivity and longevity of Pd-Fe-A and Pd-Fe-U by using the metals to reductively dechlorinate TCE (Gui and Gillham, 2002). Both complexes rapidly degraded TCE initially, but Pd-Fe-A completely deactivated within 7 days, while Pd-Fe-U remained reactive throughout the 200-day experiment (Gui and Gillham, 2002). This suggests that unwashed oxide-covered Pd plating can significantly increase the life span of ZVI. Research has yet to explore this technology on the nanoscale.

Synergistic Dechlorination by ZVI and Anaerobic Organisms

A novel idea is that the presence of nanoscale ZVI in soils or sediments could stimulate anaerobic microbial reductive dechlorination. Nanoscale ZVI instantaneously drives down the oxidation-reduction potential (ORP) of sediments upon application (Gardner, 2004). In his research, Dr. Gardner noted sediment ORPs below -600 mV (Gardner et al., 2004). Such an environment is

immensely favorable to an assortment of anaerobic organisms like sulfate reducers and methanogens. Furthermore, sulfate reducers and methanogens have been shown to use reducing equivalents resulting from iron corrosion (Rosenthal et al., 2004). A zero-valent metal with a slow deactivation rate could be used to simply drive down the ORP of contaminated sediments to incite reductive dechlorination by indigenous or augmented PCB-dechlorinating cultures.

A recent study conclusively proves that ZVI and *Dehalococcoides* spp. cooperatively dechlorinate tetrachloroethene (PCE) (Rosenthal et al., 2004). In the presence of ZVI, a mixture of two *Dehalococcoides* strains completely dechlorinated PCE to ethene within 30 days (Rosenthal et al., 2004). The two processes worked much better in conjunction than as independent reducing agents (Rosenthal et al., 2004). The ZVI promoted favorable redox conditions and served as the electron donor for reductive dechlorination by *Dehalococcoides* spp. The anaerobic corrosion of ZVI releases hydrogen at a slow rate, selecting for dechlorinating populations over methanogens (Rosenthal et al., 2004). This phenomenon might explain the extensive dechlorination demonstrated by Dr. Gardner's preliminary microscale ZVI research. The UNH project is the only study of ZVI-induced PCB reduction in contaminated sediments, and it has yielded by far the most encouraging results. Both the Housatonic River and New Bedford Harbor sediments are known to contain a plethora of dechlorinating cultures (Bedard, 2003). These organisms could have taken advantage of the low ORP and used ZVI as an electron donor ro rapidly reduce PCBs.

Enantiomeric and Isotopic Fractionation

An examination of enantiomeric and/or isotopic fractionation during synergistic dechlorination could distinguish biotic from abiotic processes (Abraham et al., 2002). Only biological processes can alter the enantiomeric properties of chiral compounds (Abraham et al., 2002). Pakdeesusuk et al. have proven that the enzymatic dechlorination of certain chiral PCBs is enantioselective (Pakdeesusuk, 2002). Evidence of enantionomeric fractionation therefore can be used to identify microbial PCB dechlorination processes (Abraham et al., 2002). Changes in the isotopic ratios of carbon ($^{13}C/^{12}C$) and chlorine ($^{37}Cl/^{35}Cl$) can indicate dechlorination mechanisms as well. The microbial dechlorination of 2,3,4,5-tetrachlorobiphenyl does not enrich the heavier ^{13}C isotope, signifying an absence of isotopic fractionation (Drenzek et al., 2001). If other congeners exhibit this behavior, the depletion of ^{13}C levels relative to manufactured values proves biotic PCB dechlorination to be prevalent (Drenzek et al., 2001). This conclusion can only be made if changes in isotopic ratios are consistent with those caused by microbial dechlorination and discernable from those caused by abiotic processes. The ubiquity of chlorine isotopic fractionation is in question. Sediments at the New Bedford Superfund Site show a significant buildup of ^{37}Cl, indicating microbial preference toward the lighter ^{35}Cl isotope (Abraham et al., 2002). Laboratory work demonstrates that the microbial dechlorination of 2,3,4,5-tetrachlorobiphenyl causes no pronounced chlorine isotopic fractionation (Drenzek et al., 2004). This report is suspect because it is highly unlikely that the widespread dechlorination in New Bedford Harbor is attributable solely to abiotic processes. Concrete ways of differentiating microbial and chemical dechlorination could assist the development of nanoscale ZVI for PCB remediation.

Technology Assessment

Nano- and potentially microscale zero-valent metals have great potential for in situ PCB remediation. ZVI oxidizes to the environmentally friendly Fe(III) and can be applied through direct subsurface injection (Gardner, 2004). Questions remain as to the effectiveness of pure nano-ZVI, but palladium coatings can catalyze dechlorination and increase ZVI longevity. Researchers should examine the potential uses of Fe(II) and Fe(III), the major results of ZVI oxidation. Fe(II) might be able to reduce PCB in its own right. Native iron-reducing bacteria could turn the evolved Fe(III) back to Fe(II) and make for a sustainable remedial cycle. The extreme oxidation state of PCB makes this idea somewhat unreasonable, though the process has been legitimately proposed for chlorinated ethenes (Wrenn, 2004). A more realistic idea is to promote iron-reducing cultures that may cometabolically dechlorinate PCBs. PCB dechlorination has been shown to occur under iron(III) reducing conditions (Wiegel and Wu, 2000). A total understanding of the fate and transport of nanoscale ZVI is necessary prior to its commercial use in soils and sediments. Mass balances and PCB-dechlorinating pathways must be confirmed, and the relationship between nanoscale ZVI and dechlorinating organisms must be studied. Yet paramount to the future of ZVI is a decreased cost of iron and palladium and an improved availability of PCBs in soils and sediments.

VIII. The "Availability" Problem

The barrier common to all of the described in situ remediation technologies is the limited availability of PCBs in soils and sediments. The hydrophobic nature of PCBs allows them to tightly adsorb to organic matrices within soils and sediments, rendering them resistant to microbial attack and chemical reduction (Mondello, 2002). There is generally a fraction of sediment-bound PCBs that readily desorbs, as well as a "slow" fraction of strongly sorbed particles (Gardner et al., 2004). The degree of sorption is dependent on the organic content of the sediment/soil (Mondello, 2002). This effect is best demonstrated by Dr. Gardner's results showing the impairment of PCB dechlorination in the clayey, organic-rich sediments of New Bedford Harbor (Gardner et al., 2004). Another study asserts that at most 60 percent of PCBs at any sediment depth are available to biological or chemical processes (Mondello, 2002). Any successful in situ remediation scheme will address the problem of PCB availability.

The most common way to increase PCB desorption is through the addition of a surfactant. Surfactants are surface acting agents that increase solubility by lowering the interfacial surface tension between aqueous and non-aqueous phase fluids (Abraham et al., 2002). Past experiments using surfactant amendments to increase PCB degradation have had mixed results (Mondello, 2002). Humic substances and most other surfactants have been found to increase PCB degradation and dechlorination yields (Fava and Piccolo, 2002), yet some surfactants adversely affect bioremediation by decreasing microbial populations (Abraham et al., 2002). Recent surfactant studies are very encouraging. Enzymatically synthesized maltotriose esters were shown to substantially increase the bioavailability of Aroclor 1242 (Ferrer et al., 2003). When incubated with LB400, Aroclor 1242-contaminated soil amended with such a surfactant showed a 92 percent

decrease in Aroclor concentration (Ferrer et al., 2003). PCB solubility was increased from 140 to 305 μg/L (Ferrer et al., 2003). Most importantly, the high degradation rates prove the surfactant was non-toxic to LB400.

It has been reported that randomly methylated-beta-cyclodextrin (RAMEB) substantially increases PCB bioavailability while simultaneously stimulating PCB-degrading aerobic bacteria (Fava et al., 2003b). Researchers treated PCB-contaminated soil with varying amounts of RAMEB in small reactors. RAMEB greatly increased the fraction of aqueous PCBs and slowly degraded in the presence of indigenous soil organisms (Fava et al., 2003b), which was very beneficial as the natural degradation of the surfactant actually promoted biphenyl- and chlorobenzoate-degrading populations. In recent work, Dr. Gardner uses ethanol to extract PCBs from sediment matrices (Gardner, 2004). Ethanol is advantageous because it is cheap and environmentally friendly (Gardner, 2004). Ethanol also acts as a solvent to release hydrogen from the Pd/Fe complex, catalyzing dechlorination. Results from Dr. Gardner's work are pending.

Electrokinetic manipulation of sorbed PCBs also can increase the contaminant's availability to microbial or chemical processes. Oxford University researchers are currently exploring the ability of electric currents to desorb PCBs and move them micrometers in soil (Jackman, 2004). Control of the induced PCB movement is essential to ensure the contaminant's bioavailability to organisms in the soil (Jackman, 2004). While some sediments or soils are more conducive to desorption, surfactants or electrodes must be used in any in situ PCB remediation scheme. The "slow" PCB fraction otherwise will persist. An efficient and environmentally friendly method of PCB manipulation must be uncovered to allow in situ eradication of the contaminant.

IX. Conclusion

Despite years of research and many promising leads, an effective in situ remediation technique for PCB-contaminated soils and sediments does not exist. A sequential anaerobic/aerobic bioremediation system has always exhibited enormous potential at the laboratory scale. Dechlorinating cultures *o*-17 and DF-1 have been identified, and research is on the verge of isolating PCB-respiring organisms (Wu et al., 2002). Ways to prime dechlorination are established, and dechlorination pathways are well known (Bedard, 2003). Aerobic strains LB400(pRO41) and RHA(pRHD34) adeptly grow on and mineralize most of the major anaerobic dechlorination products (Tiedje, 2004). Doubly *ortho* substituted congeners remain recalcitrant to the recombined strain, but genetic engineering can circumvent the problem by preventing DHBD inhibition (Dai et al., 2002). Comprehensive field-scale research must be conducted to advance bioremediation technology.

Nanosized ZVI is a proven PCB dechlorinator that works swiftly and efficiently. Most inspiring is the notion of a chemical reduction/biological oxidation sequence for complete mineralization of PCBs. Nanoscale ZVI, especially when palladized, is a voracious dechlorinator and can rapidly reduce Aroclor mixtures to congeners susceptible to aerobic degradation. The use of an

environmentally benign surfactant could greatly augment the removal efficiencies of such processes. Alternatively, "active" sediment caps can be used to dechlorinate PCBs as they desorb from sediment matrices. Such sediment caps must maintain reactivity for extended durations. Pilot- or field-scale tests of these technologies are needed to further assess their strengths and shortcomings.

A controversial barrier to the in situ remediation of PCBs yet to be addressed in this paper is the general public phobia of genetically modified organisms (GMOs) and nanotechnology. While many fears are unjustified, the use of GMOs and nanomaterials must be strictly monitored as several legitimate concerns do exist. Control mechanisms must prevent the environmental dispersion of engineered genes (Sylvestre, 2004). Active-and-passive biological containment (ABC) systems are being developed that trigger a "killing" gene in response to an environmental signal (Sylvestre, 2004). Mastery of ABC techniques might quell the public distrust of GMOs. Nanoparticles are feared to enter the food chain, self-replicate, and facilitate the dissemination of non-targeted pollutants (Masciangioli and Zhang, 2003). A better understanding of the behavior of nanomaterials in sediments and soils is necessary. Control techniques for GMOs and nano-materials are important, but public hysteria should not hinder the advancement of the most promising agents for the in situ remediation of PCB-contaminated soils and sediments.

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