



## Techniques for Bioremediating PCB Contaminated Landscapes

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Disasters such as the chemical seepage from Love Canal in the late 1970's have created a demand for protection of industrial and residential areas. This demand has led to major efforts to remediate and restore contaminated landscapes. Polychlorinated biphenyls (PCBs) have received attention as a result of incidents like Love Canal. The estimated cumulative production of these chemicals is around 630 million kilograms and their use in industrial and electrical equipment continues around the world (Hutzinger and Veerkamp 1981).

Today, many landscapes in prime industrial and residential locations are still contaminated with wastes such as PCBs. Reclaiming these lands is a national priority in the U.S. but the application of techniques to remove or degrade these chemicals has been lacking. The scope of this review is to communicate techniques that have been successful in remediating landscapes contaminated with PCBs, focusing on the ability to treat PCBs on site using bioremediation. Although far from perfected, several promising field studies have demonstrated effective removal of PCB using biodegradation.

### History

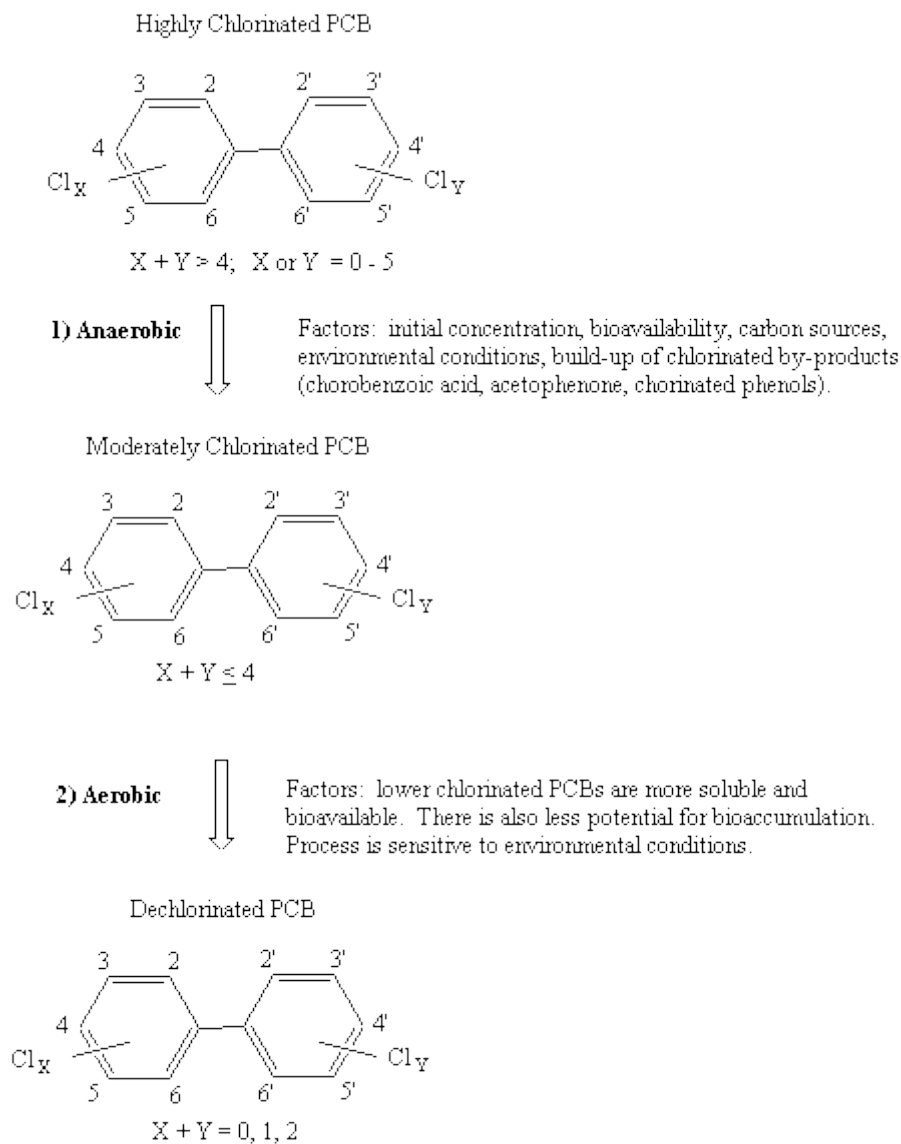
The introduction of polychlorinated biphenyls in 1929 was an industrial breakthrough that prevented disasters such as electrical fires. The mixtures of chlorinated oils that make up PCBs were found to have low reactivity, high electrical resistance, and stability under heat and pressure, making them ideal for dangerous applications including flame retardants, dielectric fluids in capacitors and transformers, and hydraulic fluids. It is the very same properties that have allowed PCBs to accumulate and persist in the environment (Boyle et al. 1992).

Although not a direct carcinogen or neurotoxin, some PCBs are highly bioaccumulative due to their low water solubility ( $\approx 10^{-5}$  to  $10^{-11}$  moles/L) and low volatilization potential (Henry's Law (dimensionless) constants  $\approx 4 \times 10^{-2}$  to  $4 \times 10^{-4}$ ) (Schwarzenbach et al. 1993). PCBs are considered potential carcinogens because some mixtures have been shown to increase the development of hepatic tumors in rats (Kimbrough 1987, Safe 1989). There are also many negative reproductive effects of PCBs on wildlife (Unterman 1995). Consequently, the U.S. Congress banned the manufacture of PCBs in 1976 (Boyle et al. 1992). However, questions still remain regarding toxicity. The Agency for Toxic Substances and Disease Registry reviewed PCB toxicity and found the available data to be inconclusive regarding PCB human exposure and neurotoxic effects (ASTDR 1993).

PCBs were manufactured as mixtures of 60 to 90 compounds with varying degrees of chlorination about a biphenyl ring (Figure 1, Schulz et al. 1989). The following nomenclature is also widely used: position 2 = ortho, position 3 = meta, position 4 = para. There are 209 theoretical combinations of chlorines arranged on one or both rings and these different chemicals are called *congeners*, or, for the same number of chlorines, *isomers*. The marketing of PCBs varied by company, resulting in several trade names: Arochlor (Monsanto, USA), Fenchlor

(Caffaro, Italy), Phenochlor and Pyralene (Prodelec, France), Clophen (Bayer, Germany), and Kanechlor (Kanegafuchi, Japan). Mixtures of PCBs are given a 4 digit code such as 1221 or 1260. The first two digits refer to the number of carbons (usually 12) and the last two digits are

Figure 1. Combined anaerobic/aerobic degradation of PCB



the average percent weight of chlorine, ranging from about 21 to 60 (Hutzinger et al. 1974).

The discovery of the biological degradation of PCBs by microorganisms was made in 1973 (Ahmed and Focht, 1973). Species of microbes were discovered by investigating known sites of PCB contamination and isolating the microbes where declines have been observed in PCB congeners.

Table I lists the sites where PCB biodegradation has been evaluated. The procedures section describes some of the techniques in more detail.

Table I. Sites of PCB biodegradation.

Site	Arochlor	Technique	respective % removal, if reported
Woods Pond, near Lenox, MA <sup>1</sup>	hexa-, hepta-, octa-chlorinated PCB	Biostimulation	70, 57, 27%
Woods Pond, near Lenox, MA <sup>2</sup>	1254, 1260	Natural Attenuation	13%
New Bedford Harbor, MA <sup>2</sup>	1242, 1254	Natural Attenuation	
Silver Lake, Pittsfield MA <sup>2</sup>	1254, 1260 (minor)	Natural Attenuation	61%
Waukegon Harbor, IL <sup>2</sup>	34-34-PCB, 234-34-PCB & 245-34-PCB	Natural Attenuation	47-98.5%, 15-89%
Sheboygan River, WI <sup>1</sup>	1248	In-situ microcosm	59%
Lake Ketelmeer, Rhine River, Netherlands <sup>2</sup>	234-34-PCB, 2345-34-PCB, 345-34-PCB, 345-345-PCB	Natural Attenuation	70-88%
Lake Shinji, Japan <sup>2</sup>	close to 1254	Natural Attenuation	
Madison Metropolitan Sewerage District, WI <sup>1</sup>	lower chlorinated PCBs, 25-25-PCB	Landfarming & Biostimulation of sewage sludge	85%, 6%
Hudson River, NY <sup>1</sup>	lower chlorinated PCBs	Aerobic biostimulation	37-55%
ALCOA <sup>1</sup>	lower chlorinated	Bio-slurry reactor	20-50%

	PCBs		
ALCOA <sup>1</sup>	lower chlorinated PCBs	Landfarming	20-50%
Pennsylvania, gas pipeline site (Envirogen, Inc.) <sup>1</sup>	lower chlorinated PCBs	Aerobic biostimulation	44%
Pennsylvania, gas pipeline site (Envirogen, Inc.) <sup>1</sup>	lower chlorinated PCBs	Bio-slurry reactor	55%
Oak Ridge Nat'l Lab <sup>3</sup>	lower chlorinated PCBs	Bioaugmentation, anaerobic & aerobic	70%
Oak Ridge Nat'l Lab <sup>3</sup>	lower chlorinated PCBs	Bioaugmentation, aerobic	67%
River Raisin, MI <sup>4</sup>	1242, 1254	Natural Attenuation	

Sources: <sup>1</sup> (Unterman 1996), <sup>2</sup>(Bedard and Quensen 1995), <sup>3</sup>(Klasson 1996), <sup>4</sup>(Mousa 1998)

The percent removal is determined by a complete congener specific analysis to determine the original content of the PCB and from the reductions in certain congeners the percent removal is calculated (Bedard and Quensen 1995).

## Procedures

The most certain way to destroy persistent chemicals such as PCBs from soils is incineration. This technique is costly in terms of transportation and energy and leaves a scarred landscape that must be either remediated or restored. Bioremediation has become a successful alternative technology because landscapes may be cleaned with much less disturbance and cost. PCBs do present a challenge to bioremediation, however, because they are so stable. Nevertheless, the potential cost savings make it a technique worthy of consideration when assessing treatment options.

Unterman describes three levels of PCB bioremediation, increasing in levels of cost and complexity (1996):

- 1) Natural Attenuation. Preexisting microbes are allowed to continue whatever degradation they can attain and the results are closely monitored.
- 2) Biostimulation. Growth of preexisting microbes is stimulated with nutrients (oxygen, carbon source, fertilizer) to increase the degradation of contaminants.

3. Bioaugmentation. Microbes with the ability to degrade PCBs are transplanted to a site along with nutrients, if necessary.

A combination of anaerobic followed by aerobic biodegradation has been found to maximize the removal of chlorine. A review of these separate pathways (six discovered so far) is beyond the scope of this paper but may be found in the literature (Unterman 1996; Bedard and Quensen 1995). The most critical stage of the two-step, anaerobic to aerobic process is getting the anaerobic bacteria to proliferate at a new site, cometabolizing the higher chlorinated PCBs (greater than 4 chlorines per biphenyl, see Figure 1). Because aerobes are only capable of degrading lower chlorinated PCBs (4 or less chlorines per biphenyl) it is important that anaerobes grow well, transforming as many of the higher chlorinated PCBs as possible to lower chlorinated PCBs. Several factors affect the growth of bacteria. Briefly, these are the chemical structure and composition of the PCB waste, the initial concentration, the solubility of the PCBs in the media, and the environmental conditions (temperature, pH, salinity, redox potential, available carbon, presence of co-contaminants). In the following section, each of these factors will be reviewed as they apply to bioremediating a site.

### **Chemical Structure and Composition**

Attention must be paid to 1) the extent of chlorination, and 2) the content of certain highly toxic congeners. It has been found that for lower weight Arochlors such as 1242 and 1248, 50 to 85% removal may be obtained using only aerobes. Thus, for mixtures of lower chlorinated PCBs, sufficient degradation may be achieved using a one-stage procedure involving only aerobes. There is a danger, however, if higher chlorinated PCBs are left intact because they are generally more bioaccumulative than the less chlorinated PCBs (Unterman 1996). Additionally, there are the highly toxic congeners referred to as "dioxin-like", containing chlorine at the two para positions (4 on the biphenyl ring) and at least two chlorines at the meta positions (3 or 5 position). The lack of ortho groups allows the atoms in these congeners to line up in a single plane (referred to as coplanar PCBs) which makes them especially toxic. Luckily, it is the meta and para positions that are most susceptible to dechlorination (Bedard and Quensen 1995).

### **Initial Concentration**

The amount of PCB present in a landscape is a factor that affects the rate of removal. It has been found that there is a threshold concentration above which dechlorination is constant. This is related to the saturation of the pore water with PCB which occurs at 300  $\mu$  g/ml of slurry or 1725  $\mu$  g/g sediment dry weight. At this concentration, the ability of microbes "to pull" the PCB out of solution becomes the rate limiting step. Below this concentration, dechlorination is linear (Bedard and Quensen 1995). Thus, the greatest rates of chlorine removal are observed at high levels of PCB contamination (typically at 1 mg/g sediment dry weight). The initial concentration also affects how much by-product will be formed (chlorobenzoic acid, acetophenone, chlorinated phenols). It has been shown that some of these by-products are capable of being biodegraded by defined cultures (Lenn 1995; Unterman 1996).

## **Solubility of PCBs in the media**

As discussed in the previous section, the maximum concentration of PCB which is able to dissolve in water (300  $\mu$  g/ml of slurry) is a limiting factor for degradation. The high hydrophobicity and low solubility of PCBs in water makes them much more likely "to stick" on soils with organic matter than in groundwaters. In addition to solubility, the term bioavailability is used throughout the literature and refers to the degree to which a chemical may be removed from solution and/or desorbed and then metabolized by biota. For PCBs, this is critical because they sorb so tightly to organic matter, becoming unavailable for uptake in some cases. Surfactants have been introduced at some field sites without success in an attempt to enhance bioavailability of PCBs (Bedard and Quensen 1995). The characterization of media is critical since a site with large amounts of organic matter and other stable oils may serve as a sink for PCBs, making them unavailable to microbes.

## **Environmental Conditions**

Perhaps the most uncertainty in determining the success of a microbial population at a new site involves the wide range of chemical and physical parameters to which microbes may be unaccustomed. Furthermore, optimal conditions (pH, temperature, salinity, sources of carbon, presence of co-contaminants, redox conditions) are often undefined for a particular microbial pathway (Bedard and Quensen 1995). Studies so far have taken place in laboratory and/or in-situ sediment slurries where control of numerous variables is difficult and time consuming. Because the study sites all vary in environmental conditions, there is no defined medium for the development of microbes nor have there been successful isolations of the microbial populations (Bedard and Quensen 1995).

Briefly, some of the successful attempts at optimizing conditions for degradation have included:

- 1) Carbon additions in the form of brominated biphenyls to stimulate the growth of microbes capable of degrading PCBs.
- 2) Addition of cultures of microbes capable of removing transformation products of PCB degradation such as chlorobenzoic acid, acetophenone, and chlorinated phenols.
- 3) Co-contaminants. No detrimental effects on the percent removal were observed at Silver Lake with levels of oil at 6-10% by weight. Likewise, no detrimental effects were found at New Bedford Harbor in the presence of chromium, cadmium, lead, and zinc (all at several hundred  $\mu$  g of metal /g sediment). The effects of polyaromatic hydrocarbons and other halogenated compounds are unknown (Bedard and Quensen 1995).

## **Conclusion**

It has been shown that PCBs can be degraded in-situ by anaerobic and aerobic processes. Bioremediation of these recalcitrant chemicals presents one of the more difficult applications for

microbes because of the high stability of PCBs. Although commercial technology has not been marketed for the bioremediation of PCBs, it is being field tested in the U.S. by General Electric, ALCOA, Envirogen Inc., Oak Ridge National Laboratory, U.S. EPA, and several academic institutions. Because of the large number of variables affecting PCB biodegradation, thorough characterization of a PCB waste and the media is particularly important when assessing options for treatment.

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