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Aerobic and Anaerobic Biodegradation of PCBs: A Review

Daniel A. Abramowicz

ABSTRACT

This review summarizes recent research results on the biodegradation of polychlorinated biphenyls (PCBs). These compounds, commonly believed to be indestructible, have repeatedly been shown to biodegrade under a variety of conditions. Two distinct classes of bacteria have now been identified that biodegrade PCBs by different mechanisms. The focus of this manuscript is current research involving the aerobic biodegradation of PCBs (natural strains, recombinant organisms, and soil applications) and the dramatic new results demonstrating microbial reductive dechlorination of even highly chlorinated PCBs under anaerobic conditions.

These two PCB-degradative systems include aerobic bacteria which live in oxygenated environments and anaerobic bacteria which live in oxygen free environments such as aquatic sediments. The aerobes attack PCBs oxidatively, breaking open the carbon ring and destroying the compounds. Anaerobes, on the other hand, leave the biphenyl rings intact while removing the chlorines. This anaerobic dechlorination degrades highly chlorinated compounds into less chlorinated derivatives. These two naturally occurring processes are complementary, and a two step treatment may permit the biological destruction of nearly all of the PCB mixtures commonly used.

I. INTRODUCTION

A. Definition

Polychlorinated biphenyls (PCBs) are a family of compounds produced commercially by the direct chlorination of biphenyl using ferric chloride and/or iodine as the catalyst.[51] The biphenyl molecule is made up of two connected rings of six carbon atoms each (see Figure 1), and a PCB is any molecule having multiple chlorines attached to the biphenyl nucleus. Chlorines can be placed at any or all of the ten available sites, with 209 different PCB compounds theoretically possible, varying in the number and position of the attached chlorines. The individual isomers and homologs are generically referred to as congeners. Of the 209 possible congeners, only about half are actually produced in the synthesis due to steric hindrance. The position of the chlorines is indicated by the numbering scheme shown in Figure 1. The reaction shown in Figure 1 would produce a large number of different PCB structures; only the 2,3,4,3′,4′-pentachlorobiphenyl (2,3,4,3′,4′-CB) is drawn as an illustration.

PCBs were manufactured and sold as complex mixtures differing in their average chlorination level. The crude mixtures

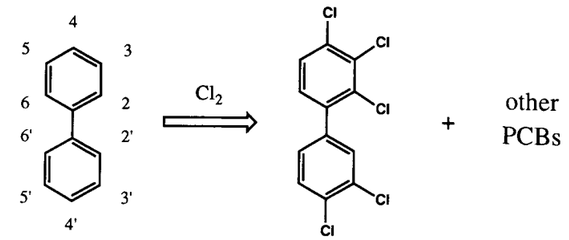

FIGURE 1. The synthesis of PCBs via the direct chlorination of biphenyl. Note that this method produces nearly 100 different PCB products, with the congener 2,3,4,3′,4′-CB included as an example.

resulting from the chlorination were fractionally distilled to produce commercial mixtures with the desired properties. The products range from light oily fluids (di-, tri-, and tetra-chlorobiphenyls) to heavy, honey-like oils (penta-chlorobiphenyls), to greases and waxes (more highly chlorinated). The manufacturers of PCBs sold the materials under various trade names: "Aroclor" (Monsanto, U.S.); "Phenoclor", and "Pyralene" (Prodelec S.A., France); "Clophen" (Farbenfabriken Bayer AG, Germany); and "Kanechlor" (Kanegafuchi Chemical Industrial Co. Ltd, Japan). The manufacturers also assigned product numbers that usually reflected the degree of chlorination by either the average number of chlorines/biphenyl or the weight percent chlorine in the mixture. For example, Aroclor 1242 (12 carbon atoms and 42% chlorine), Clophen A 30 (3 chlorines/biphenyl), and Kanechlor 300 (3 chlorines/biphenyl) all contain 42% chlorine by weight which corresponds to three chlorines/biphenyl on average. Likewise, Aroclor 1260 and Clophen A 60 contain 60% chlorine and 6 chlorines/biphenyl on average.

B. Properties

The desirable physical and chemical properties of PCBs led to their widespread use. The most important physical properties of the mixtures are that they are liquids, have low vapor pressures, low water solubility, and excellent dielectric properties. Chemical properties include stability to oxidation, flame resistance, and relative inertness. Because of excellent flam-

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mability, electrical, and stability properties, PCBs found application in a wide variety of industrial uses including heat transfer fluids, hydraulic fluids, solvent extenders, plasticizers, flame retardants, organic diluents, and dielectric fluids.[51]

In a 50-year period approximately 1.4 billion pounds of PCBs were produced. Such extensive application of these chemically and thermally stable compounds has resulted in widespread contamination.[20,52,88] It is estimated that several hundred million pounds have been released to the environment.[50] The lipophilic nature of PCBs contributes to their tendency to accumulate in fatty deposits and results in a magnification in the food chain.[80]

C. Health Risk

This accumulation of PCBs in organisms and the past exposure of some industrial workers was initially a cause for concern.[55,71] But the toxicity associated with PCBs has recently been re-evaluated.[30,57,58,64] It has now been concluded that " . . . the only observed acute effects have generally been minor. So far, no significant chronic health effects have been causally associated with exposure to PCBs or PBBs."[58]

Another health risk commonly associated with PCBs involves their role as suspected human carcinogens. This premise stems from early reports that high levels of Aroclor 1260 caused liver cancer in rats.[56] But a study by the National Cancer Institute (1978) concluded that Aroclor 1254, a mixture of PCBs having a slightly lower level of chlorination than Aroclor 1260, was not carcinogenic.[70] In addition, a recent thorough review of the epidemiological literature stated that "No conclusive evidence thus far reported shows that occupational exposure to PCBs causes an increased incidence of cancer."[58]

Most reviews concerning the biological and toxic effects of PCBs note that the relative potency generally correlates with the degree of chlorination.[32,55] These results suggest that the toxicities of the mixtures are variable, and it is therefore reasonable that the activities of individual congeners may also differ considerably. Valuable data involving structure/activity relationships for individual congeners is now available.[75,81,82] Safe has concluded from animal studies carried out in his laboratories that the most toxic PCB congeners contain two *para* and at least two *meta* chlorines, and the addition of *ortho* chlorines reduces this effect significantly.[81]

II. AEROBIC BIODEGRADATION OF PCBs

A. Enrichments

Most of the environmental contamination by PCBs is in the form of complex commercial mixtures (e.g., Aroclor 1242) containing >60 different congeners with varying degrees of chlorination. Biodegradation of this large number of distinct substrates therefore requires broad enzymatic specificity. In addition, chlorinated organic materials frequently resist microbial degradation.[6] Although these complex chlorinated mixtures can be difficult to biodegrade, the aerobic bacterial biodegradation of PCBs is known and has been well studied.[5,10,11,14,39,40,59,62,66,73,86] Previous reviews on the aerobic biodegradation of these materials have been published,[41,42] and this review concentrates on research results reported after their publication.

Using a rapid screening procedure, Bedard et al.[9] isolated natural aerobic bacteria capable of degrading PCBs in nearly every contaminated soil they tested. Soil and sediment samples were collected from PCB-contaminated sites and cultures were enriched on biphenyl as the sole carbon and energy source available to the bacteria. The bacterial enrichments obtained were assayed for their ability to degrade defined mixtures of PCBs. Using this approach, a diverse group of 25 strains of PCB degrading bacteria were isolated and characterized.[9,92] This method allowed the rapid determination of PCB competence for a large number of isolates. In addition, the use of defined PCB mixtures in place of complex Aroclors permitted investigations into the nature of the enzymatic specificity observed. The results of this screening technique are shown in Figure 2.[1,9] Note that all of the organisms isolated are capable of degrading the lightly chlorinated PCBs. Characterization (genus and species) for some of the PCB degrading organisms isolated by several different workers is shown in Table 1. These results indicate that naturally occurring organisms can degrade PCBs, are quite common in the environment, and that the organisms consist of many different microbiological types. It is interesting to note that nearly two-thirds of the organisms represented in this survey are members of the genus *Pseudomonas*.

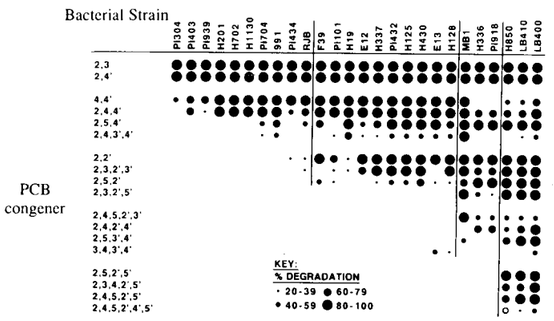

FIGURE 2. Comparison of the PCB-degrading competence of environmental bacterial isolates. [○] indicates that H850 degraded less than 20% of this congener (2,4,5,2′,4′,5′-CB), but a metabolite was isolated. (Adapted from Bedard, D. L., Unterman, R., Bopp, L. H., Brennan, M. J., Haberl, M. L., and Johnson, C., *Appl. Environ. Microbiol.*, 51, 761, 1986 and Abramowicz, D. A., *Hazardous Waste Treatment: Biosystems for Pollution Control*, Air and Waste Management Assoc., Pittsburgh, 1989, 301. With permission.)

B. Metabolic Pathway

The actual biochemical steps involved in the aerobic biodegradation of PCBs have been previously determined. In general, attack involves initial addition of O_2 at the 2,3- position by a dioxygenase enzyme, with subsequent dehydrogenation to the catechol followed by ring cleavage (see Figure 3).

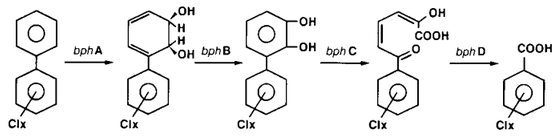

FIGURE 3. Degradation of biphenyl and chlorobiphenyls by the 2,3-dioxygenase pathway in *Pseudomonas* strain LB400. Gene designations: *bphA*, biphenyl 2,3-dioxygenase; *bphB*, dihydrodiol dehydrogenase; *bphC*, 2,3-dihydroxybiphenyl dioxygenase; *bphD*, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (*meta*-cleavage product) hydrase. (From Mondello, F. J., *J. Bacteriol.*, 171, 1725, 1989. With permission.)

This pathway is similar to the degradation pathways for other aromatic substrates deduced for biphenyl[47] and for toluene.[34] The first two steps in the metabolism of biphenyl involve dioxygenase attack at the 2,3- position with subsequent dehydrogenation to the catechol.[47] The next step involves fission of the ring to the *meta*-cleavage product.[25] These authors also proposed that this ring fission product was further metabolized to benzoic acid, as this metabolite was identified from crude cell-free mixtures incubated with 2,3-dihydroxybiphenyl. This cleavage to benzoic acid was later confirmed.[72]

In the earliest reported isolation of PCB-degrading strains, Ahmed and Focht[5] identified both the *meta*-cleavage product and *p*-chlorobenzoic acid as metabolites of the degradation pathway. These authors postulated that the PCB degradation pathway is the same as that determined earlier for biphenyl and other aromatic hydrocarbons. This hypothesis was confirmed by Furukawa et al.[38] with the identification of the *meta*-cleavage product and chlorobenzoic acids as metabolites of PCBs.

In general, most PCB degrading aerobic bacteria are able to degrade only the lower chlorinated PCB congeners (e.g., mono- to tetra- substituted).[5,7,39,91] It is possible that higher chlorination levels result in steric hindrance of 2,3-dioxygenation by chlorine substitution at either of these two positions.[74] But several aerobic bacterial strains have demonstrated the exceptional ability to degrade an even larger range of congeners, up to and including penta-, hexa-, and even several heptachlorobiphenyls (*Pseudomonas* strain LB400,[14] *Alcaligenes eutrophus* H850,[10,11] *Corynebacterium* strain MB1,[10,12] and *Acinetobacter* strain P6.[39,40] One of these organisms has demonstrated the capacity to degrade more than 90% of the PCBs present in the mixture Aroclor 1242 (LB400).[66]

Although these organisms use the 2,3-dioxygenase degradative pathway described above, it is possible that PCBs are also metabolized through other routes. It is known that congeners containing a 2,5-chlorophenyl ring are preferentially degraded by strains H850[11] and LB400.[14] In addition, the production of different metabolites led to the proposal that a significant mechanism for PCB metabolism in these organisms involves a novel 3,4-dioxygenase attack.[9,11] This proposed 3,4-dioxygenase attack has been confirmed by Gibson[69] in both H850 and LB400 by identification of the expected *cis*-dihydrodiol intermediate from 2,5,2′,5′-CB. This additional dioxygenase pathway may partially explain the exceptional range of PCB-degrading activity demonstrated by *A. eutrophus* H850 and *Pseudomonas* sp. LB400.

It is not currently known if the 2,3- and 3,4-dioxygenase activities originate from the same enzyme. It is clear, however, that the congener specificity indicates two distinct classes of dioxygenases. The dioxygenase type present in *Acinetobacter* P6 and *Corynebacterium* MB1 is particularly active against congeners containing double *para*- substitution, while the enzyme from *Alcaligenes* H850 and *Pseudomonas* LB400 prefers 2,5- substitution patterns. In general, these specificities are complementary and treatment with an organism from each class results in even greater PCB degradation.[92]

C. Optimization

It has been demonstrated that growth on biphenyl as the sole carbon source is required for optimal PCB degradative activity (LB400).[66] This is a disadvantage in soil applications where other carbon sources are available. The degradation of PCBs bound to soil has been investigated.[63,92] Although PCBs are degraded in these systems, the rates decrease significantly (more than 50-fold) compared to the biphenyl assays. One possible explanation is that biphenyl is required as the sole carbon source for maximal induction of the PCB degrading enzymes. The importance of biphenyl in the soil degradation of PCBs has been investigated by Focht[19,35] and enhanced degradation of

Table 1
Partial Listing of Aerobic Environmental Isolates Capable of PCB Biodegradation

Organism	Strain designation
Achromobacter sp.	BP, pCB
Acinetobacter sp.	P6, LS241
Alcaligenes sp.	KF708, Y42,BM—2
Alcaligenes eutrophus	H850
Alcaligenes faecalis	Pi434
Arthrobacter sp.	M5, B1B
Corynebacterium sp.	MB1
Pseudomonas sp.	LB400, LB410, KF714, JB1, IS140, 7509, WR912
Pseudomonas (*Acidovorans* group)	Pi939, H1130, Pi304, H702, Pi101
Pseudomonas cepacia	H201, Pi704, RJB
Pseudomonas paucimobilis	Q1
Pseudomonas pseudoalcaligenes	KF707
Pseudomonas putida	KF715, OU83
Pseudomonas testosteroni	H128, H336, H430

PCBs on soil were observed upon the addition of biphenyl as a carbon source. In addition, the PCB-degrading activity of growing cells was significantly greater for *Acinetobacter* sp. P6 and *Arthrobacter* sp. B1B than the activity observed with resting-cell suspensions.[58a] Biphenyl was utilized as the carbon source, and it is reasonable to conclude that biphenyl is required for maximal PCB degradative competence as an inducer of this dioxygenase pathway.

The PCB degradation pathways described earlier produce chlorobenzoates that are not further metabolized by these strains, although other organisms are known to mineralize these compounds.[29,83] Although many organisms can grow on monochlorobiphenyls (LB400, H850, KF715, KF707, KF708, Q1, M5, BM-2, MB1), microorganisms which could use complex PCB mixtures as a carbon source may perform better in soil applications. Strains which can degrade monochlorobiphenyls and further metabolize the chlorobenzoates have been reported. A *Pseudomonas* strain JB1 was isolated that can grow on monochlorobiphenyls, degrade mono-chlorobenzoates, and can cometabolize other congeners.[74] In addition, Focht and Huang[36] have developed a new strain which is also capable of degrading monochlorobiphenyls and metabolizing the chlorobenzoate intermediates, resulting in growth on 3-CB as the sole carbon source. This strain was generated via a method that facilitates the rapid exchange of genetic material between two parent strains. The development of new strains that could grow on the more highly chlorinated PCBs would represent a major advance in the aerobic biodegradation of PCBs.

Other methods to enhance the aerobic bacterial biodegradation of PCBs have also been reported. The addition of the aminopolysaccharide polymer chitin has been observed to increase the rate of PCB degradation.[76,77] The effects of polymer addition are shown in Table 2. Note that this method generally resulted in a twofold increase in the degradation rate by the indigenous soil microorganisms. The chitin appears to act as a solid substrate for growth as well as an efficient sorbing component for the PCBs, and therefore increases the bioavailability of these hydrophobic compounds. The addition of adapted PCB degrading bacteria resulted in even greater soil degradation rates (Table 2, microbe addition).

Table 2
Half-Life Estimates for Specific PCB Congeners with and without the Addition of Chitin or Chitin Plus Adapted Microbes

Toxicant	Polymer	Microbe	Half-life (days)
4,4′-CB	−	−	1.42 +/− 0.41
4,4′-CB	+	−	0.98 +/− 0.21
4,4′-CB	+	+	0.46 +/− 0.33
2,4,5,2′,5′-CB	+	−	1.32 +/− 0.4
2,4,5,2′,5′-CB	+	+	0.80 +/− 0.7
Aroclor 1232	−	−	61.4 +/− 3.6
Aroclor 1232	+	−	33.4 +/− 0.9
Aroclor 1232	+	+	26.8 +/− 0.7
Aroclor 1248	−	−	77.6 +/− 8.2
Aroclor 1248	+	−	38.6 +/− 2.4
Aroclor 1248	+	+	31.9 +/− 3.6
Aroclor 1254	−	−	81.9 +/− 7.2
Aroclor 1254	+	−	36.4 +/− 3.8
Aroclor 1254	+	+	35.5 +/− 2.2

From Portier, R. and Fujisaki, K., *Aquatic Toxicology and Hazard Assessment*, Vol. 10, ASTM STP971, Adams, W. J., Chapman, G. A., Landis, W. G., Eds., ASTM, Philadelphia, 1988, 517. With permission.

D. Genetic Engineering

The genes encoding bacterial degradation of PCBs have been isolated and utilized to construct recombinant organisms capable of degrading PCBs.[43,54,66] These studies utilized soil microorganisms from the genus *Pseudomonas* that degraded PCBs via the 2,3-dioxygenase pathway discussed earlier (see Figure 3). Furukawa and Miyazaki obtained the genes from *P. pseudoalcaligenes* strain KF707,[43] an organism known to degrade mono- to tri-CB, including 4-CB, 2,3-CB, 3,4-CB, 2,4′-CB, 2,4,5-CB, and 2,4,4′-CB. The genes were then cloned into a broad-host-range plasmid, and a transformant was isolated that was capable of degrading PCBs.[43] The researchers discovered that the genes encoding three of the four enzymes involved in PCB degradation (*bphA* through *bphC*) were localized on a small DNA fragment (7.9 kb). In addition, the 2,3-dihydroxybiphenyl dioxygenase (*bphC*) was isolated[44] and sequenced from two different organisms.[45,87] Mondello obtained the PCB-degradative genes from a *Pseudomonas* strain LB400,[66] an organism known to degrade mono- to hexa-CB, including 2,3-CB, 2,4′-CB, 2,2′-CB, 2,4,4′-CB, 2,5,2′-CB, 2,3,2′,5′-CB, 2,4,2′,4′-CB, 2,4,5,2′,5′-CB, 2,3,4,2′,5′-CB, and 2,4,5,2′,4′,5′-CB. The genes were then cloned into a broad-host-range vector, and a number of *E. coli* transformants capable of degrading PCBs were isolated.[66] All four of the PCB-degradative genes were isolated on a 12.4 kb DNA fragment, and one recombinant strain FM4560 demonstrated a PCB competence remarkably similar to the wild-type LB400 (see Figure 4). Note that the same congeners that are slowest to degrade in the wild-type organism display comparable kinetics in the recombinant organism. This similarity requires that the enzymes are expressed, functional, and catalyze reactions with the same congener specificity in the *E. coli* recombinant and *Pseudomonas* wild-type organisms. This somewhat unexpected result suggests that the PCB-degradative genes may be functional in a broad range of different microorganisms. Kahn and Walia obtained the PCB degradative genes from a *Pseudomonas putida* strain OU83 and localized the *bphC* and *bphD* genes onto a 2.4 kb DNA fragment.[54] The authors determined that the amount of *bphC* produced in the recombinant *E. coli* strain was 20-fold greater than that measured in the parent strain.

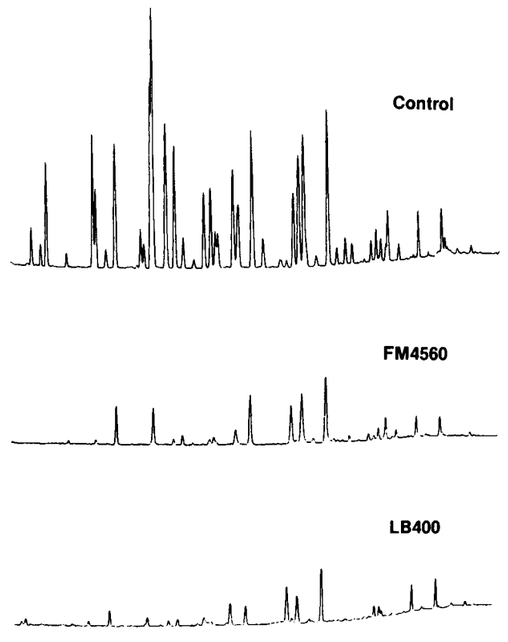

FIGURE 4. Biodegradation of Aroclor 1242 by *E. coli* FM4560 and *Pseudomonas* strain LB400. (Top) Aroclor 1242 incubated with mercury-killed cells; (middle and bottom) Aroclor 1242 (10 ppm) incubated at 30°C for 24 h with cells (optical density at 615 nm of 1.0) of FM4560 and LB400, respectively. FM4560 was grown on succinate; LB400 was grown on biphenyl. (From Mondello, F. J., *J. Bacteriol.*, 171, 1725, 1989. With permission.)

The correspondence between the PCB-degrading organisms KF707 and LB400 includes a similar metabolic pathway and similar gene organization. In addition, DNA hybridization studies revealed that the PCB-degrading genes in two very different organisms (*Pseudomonas* sp. LB400 and *Alcaligenes eutrophus* H850) were strongly conserved.[94] Additionally, the *bphABC* cluster has now been observed in five *Pseudomonas* strains and one additional *Alcaligenes* strain.[46] The high correspondence among all of these distinct organisms implies that the PCB-degradative genes did not evolve independently and the genes must have been acquired through some form of DNA transfer. This result may have important implications as it demonstrates that natural organisms can transfer and have transferred the PCB-degradative genes in the environment. These mobile genes enable the organisms to attack a broad range of PCB congeners.

Although the recombinant FM4560 can degrade PCBs no better than the wild-type LB400 under the conditions described in Figure 4, it may afford unique advantages in soil remediation applications. In addition to faster growth rates to higher cell densities, the recombinant demonstrates superior viability and temperature resistance.[65] Indeed, recombinant *E. coli* strains have been reported to survive 27 d on soils,[27] significantly better than the 2 d survival observed in the field with LB400.[63] More important is the fact that FM4560 does not require growth on biphenyl as the sole carbon source for optimal PCB-degradative competence.[66] Therefore, on soils where other organic material is readily available, the recombinant should display superior PCB-degradative competence.

E. Fungi

Microorganisms other than the bacteria, notably fungi, have also been reported to aerobically degrade PCBs. The filamentous fungus *Aspergillus niger*, used as a model of mammalian aromatic hydroxylation, has been shown to degrade the lower chlorinated PCBs in the commercial mixture Clophen A 30.[28] The wood-decay white-rot fungus *Phanerochaete chrysosporium* has also been utilized in the degradation of PCBs at very low concentrations.[21,22,31] It is believed that the same enzymes involved in lignin degradation are responsible for attack on the PCBs through the production of hydroxy radicals. This reactive intermediate should react with a wide number of organic compounds and *P. chrysosporium*, as well as other wood-decaying fungi, have been extensively studied in the degradation of a range of chlorinated organic compounds, including lindane, DDT, and chlorinated dioxins, with mineralization to $^{14}CO_2$ as the assay. The application to PCBs has demonstrated mineralization of even highly chlorinated congeners, including 3,4,3′,4′-CB,[22] 2,4,5,2′,4′,5′-CB,[21] and the mixture Aroclor 1254.[31] The results indicate that *P. chrysosporium* is capable of the complete degradation of highly chlorinated PCBs, but activity has only been observed at very low concentrations (250 ppb Aroclor 1254,[31] 5.5 ppb or 19 n*M* 3,4,3′,4′-CB).[22] Similar activities on highly chlorinated congeners have been observed with the aerobic bacteria previously described, but at much higher concentrations (10 ppm Aroclor 1254 with H850,[10] 1.8 ppm or 5 μ*M* 2,4,5,2′,4′,5′-CB with LB400,[14] and 15 ppm or 50 μ*M* 3,4,3′,4′-CB with P6.[40] The successful application of white-rot fungus to the biodegradation of PCBs will require demonstrated activity at the 50 to 1000 fold higher concentrations currently handled by bacterial systems.

F. Summary

A large number of naturally occurring, aerobic microorganisms have been isolated from many different locations and studied for their ability to degrade PCBs. The organisms range from common soil bacteria to more complex fungi. Some of the major findings follow. (1) Most soils contaminated with PCBs contain organisms with some level of PCB-degrading ability. (2) These microorganisms display congener specificity and therefore degrade individual congeners at different rates. (3) Most aerobic bacteria that have been isolated degrade only the lightly chlorinated congeners, although some bacteria have been isolated that are capable of attacking congeners containing

as many as seven chlorines. (4) For the known cases, the 2,3-dioxygenase pathway is common and quite similar in otherwise unrelated organisms. (5) Similarities in the genes encoding PCB degradation imply that these genes are being transferred between bacteria in the environment. (6) In general, the effect of aerobic bacterial PCB biodegradation is to remove the less chlorinated congeners. (7) No aerobic microorganisms have been reported that degrade the more highly chlorinated commercial mixtures Aroclor 1260 or Clophen A 60.

III. ANAEROBIC BIODEGRADATION OF PCBs

A. Environmental Evidence

Despite the extensive research on the aerobic biodegradation of PCBs, little was known about their fates in anaerobic environments such as river or lake sediments until very recently. Early studies indicated that anaerobic fermentations did not alter PCB concentrations with organisms from silage[37] or marine sediments.[24] But more recently, alterations of the PCBs present in anaerobic river and lake sediments have been observed.[15-17] These alterations involve the extensive removal of highly chlorinated PCB congeners with corresponding increases in congeners containing only a few chlorines (mono- and dichlorobiphenyls).

Several different patterns or alterations were observed for Hudson River sediments originally contaminated with Aroclor 1242 (see Figure 5). All three patterns showed markedly lower levels of most tri-, tetra-, and pentachlorobiphenyls and increased levels of mono- and dichlorobiphenyls.[16] Note that the detector response displayed in the chromatogram is non-linear and particularly poor for the congeners containing very few chlorines with short elution times.[68] Quantitation of the individual capillary chromatogram peaks indicated that in all sediments the levels of 2,6,2′-, 2,6,3′- and all dichlorobiphenyls increased 2- to 6-fold, and the level of the monochlorobiphenyl 2-CB increased 7- to 70-fold.

The observed transformations are congener specific, demonstrating selective removal of *meta* and *para* chlorines and increases in the expected partially dechlorinated PCB congeners. No known transformation processes such as evaporation or aerobic degradation could account for the striking changes observed, and it was therefore proposed that anaerobic microorganisms present in the sediments were reductively dechlorinating the PCBs.[15] In addition, transformation of even the highly chlorinated Aroclor 1260 had been observed in the environment.[16]

Anaerobic dechlorination of chlorinated aromatic compounds is not unprecedented. Tiedje and coworkers identified an anaerobic sulfidogenic bacterium strain DCB-1 capable of reductively dechlorinating dichlorobenzoates.[85] This organism represents the first and only anaerobe in pure culture capable of aromatic reductive dechlorination. It was isolated from an

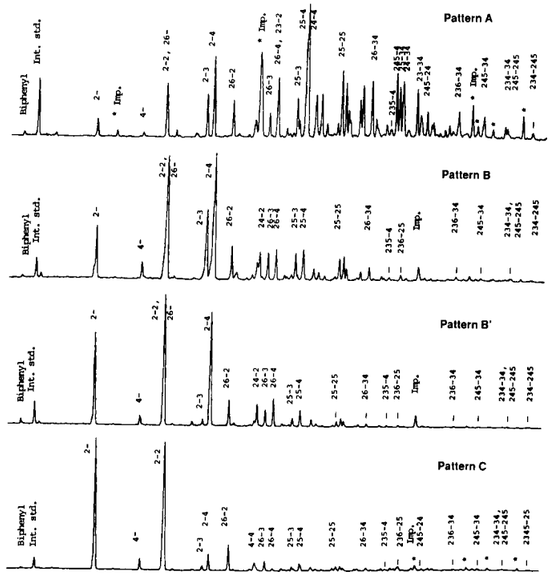

FIGURE 5. DB-1 Capillary chromatograms (plots of detector response vs. elution time) of upper Hudson River sediments that show surface pattern A (largely unchanged Aroclor 1242) and subsurface patterns B, B′, and C. A flame ionization detector was used so that PCB peak response was nearly proportional to molar concentration, however non-PCB impurities also produced observable peaks (designated * and "Imp."). (From Brown, J. F., Jr., Bedard, D. L., Brennan, M. J., Carnahan, J. C., Feng, H., and Wagner, R. E., *Science*, 236, 709, 1987. With permission.)

anaerobic consortium capable of mineralizing chlorobenzoates.[83] A review of the anaerobic dehalogenation of pesticides has recently been completed.[61] This review discusses the dechlorination of a number of aromatic substrates, including chlorobenzoates, chlorophenols, chloroanisoles, and herbicides. Reductive dechlorination of aromatics has also been reported with aerobic bacteria (chlorinated phenols and chlorinated quinones).[48,49]

B. Laboratory Confirmation

The proposed microbial dechlorination in anaerobic river sediments was confirmed in the laboratory.[78] The result of anaerobic dechlorination of Aroclor 1242 by microorganisms in Hudson River sediments is shown in Figure 6. Note the dramatic loss of the highly chlorinated congeners with corresponding increases in the less chlorinated products. These microorganisms dechlorinate the PCB mixture so extensively that it is converted from 85% tri- and tetra-chlorinated PCBs to 88% mono- and dichlorinated products. The end result of this natural process is the conversion of the more highly chlorinated PCBs into congeners of low toxicity that are degraded by a large number of aerobic bacteria.

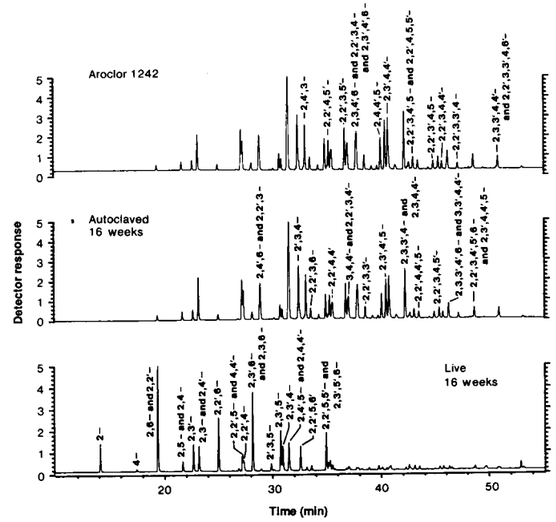

FIGURE 6. Capillary gas chromatograms showing the anaerobic dechlorination of Aroclor 1242 by Hudson River microorganisms. All chromatograms were normalized so that the highest peak had a height of 5. An electron capture detector was used. From Quensen, J. F., III, Tiedge, J. M., and Boyd, S. A., *Science*, 242, 752, 1988. With permission.)

The dechlorination was found to represent selective removal of *meta* and *para* chlorines as well, confirming that this natural process observed in the lab is the same as the dechlorination found in the environment. Therefore an additional benefit of this anaerobic dechlorination is the removal of the *meta* and *para* chlorines known to contribute to PCB toxicity.[81] The similarity between environmental and laboratory changes can be seen by comparing Figures 5 and 6.

Methods to accelerate this desirable natural process have also been identified.[2,4] The addition of a simple minimal medium (first described by Shelton and Tiedje)[84] containing nutrients and trace minerals results in a significantly more rapid activity compared to that of unsupplemented sediments. Other factors which increase the rate of dechlorination include the addition of a complex carbon source (fluid thioglycollate medium with beef extract) or a detergent (Triton X-705).[2] These effects are additive and a combination of variables results in even greater enhancement. Importantly, the dechlorination of the highly chlorinated Aroclor 1260 has also been observed in the laboratory.[4,78a] The concentration of the most highly chlorinated congeners (hexa-, hepta-, and octachlorobiphenyls) has been decreased by more than one-third the original level in Aroclor 1260.[4] Anaerobic dechlorination of Aroclor 1260 has recently been observed by others as well.[8,67,90]

C. Single Congener

Methods for the synthesis of individual PCB congeners[23] and commercially available material have provided single compounds for a more detailed study of this anaerobic dechlorination process.[3,4] The timecourse of the dechlorination observed with Hudson River sediments on the congener 2,3,4,3′,4′-CB is shown in Figure 7. The dechlorination activity observed demonstrates a sequential pathway from the penta- (2,3,4,3′,4′-CB) to tetra-(2,4,3,′,4′-CB), tri- (2,4,3′-CB), di-(2,3′-CB), and mono- (2-CB) chlorinated biphenyls (major products shown in parentheses). The result of this process is the conversion of one of the more toxic congeners (2,3,4,3′,4′-CB) into a monochlorobiphenyl (2-CB) which has low toxicity and is easily metabolized by aerobic bacteria and higher organisms.

This result again confirms that chlorines are removed from only the *meta* and *para* positions as was observed for the river sediment itself.[16] The selectivity of each individual step is surprising; for example 2,4,3′,4′-CB is the only detectable tetrachlorinated product observed from 2,3,4,3′,4′-CB, and it is produced in stoichiometric amounts.

This microbial selectivity for only *meta* and *para* chlorines is different than that observed from the direct electrochemical reduction of PCBs. Farwell et al.[33] determined that although the PCB reduction pathways were complex, dechlorination via voltammetric reduction was observed from the *ortho*, *meta*, and *para* positions. In some cases the *ortho* dechlorinated species was the major product, for example 2,3-CB yields 89% 3-CB and only 11% 2-CB.[33] This can be contrasted to the microbial dechlorination of 2,3′-CB, where 2-CB is the only observable product.[4]

The dechlorination of single congeners with higher toxicity has also been demonstrated by Tiedje et al.[89] These investigators found that 2,3,4,3′,4′-CB and 3,4,3′,4′-CB were dechlorinated at rates comparable to other penta- and tetrachlorobiphenyls, even in the presence of the complex PCB mixture Aroclor 1242.

From such studies utilizing single PCB congeners, one can prove that microbial reductive dechlorination is occurring in the sediments. The stoichiometric production of PCBs containing fewer chlorines demonstrates the substitution of hydrogen in place of the chlorine. It is believed that the anaerobic microorganisms are utilizing the chlorine as the terminal electron acceptor, involving the addition of the electron to the carbon-chlorine bond, followed by chloride loss and subsequent hydrogen abstraction (see Figure 8). The compound from which the hydrogen is ultimately abstracted is unknown, and potential primary electron donors include water, hydrogen, or an organic compound. The availability of hydrogen in anaerobic microbial systems may make it the most likely primary source of reducing equivalents.

Recently it has been shown by Hogenkamp and coworkers[60] that vitamin B_{12} can catalyze the reductive dechlorination of carbon tetrachloride and other chlorinated methanes. Vitamin B_{12} is a known hydride transfer agent and this result suggests an alternative dechlorination mechanism involving a single step

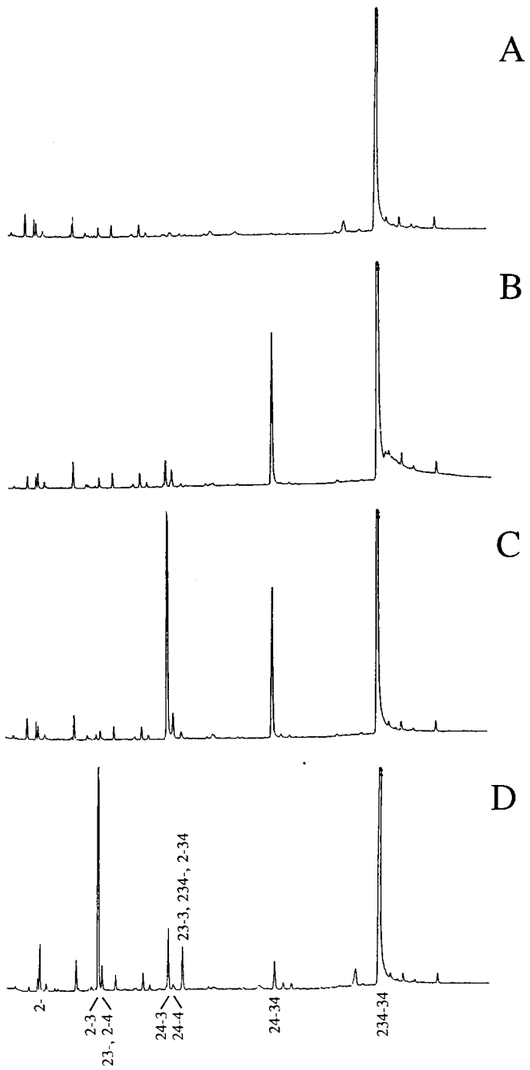

FIGURE 7. Stepwise dechlorination of 2,3,4,3′,4′-CB (500 ppm) by anaerobic Hudson River sediments. Panel A, autoclaved control; Panel B, 4 weeks; Panel C, 8 weeks; Panel D, 12 weeks. From Abramowicz, D. A., Brennan, M. J., Van Dort, H. M., and Gallagher, E. L., *Chemical and Biochemical Detoxification of Hazardous Waste II*, Glasser, J., Ed., Lewis Publishers, Chelsea, MI, 1990. With permission.)

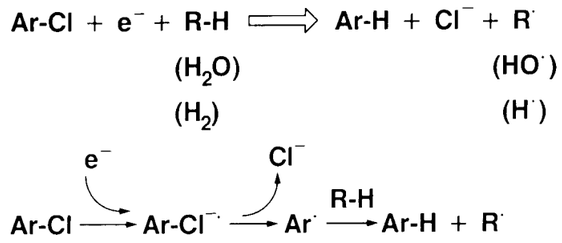

FIGURE 8. Possible mechanism for reductive dechlorination catalyzed by anaerobic microorganisms. In the proposed scheme, the organisms utilize PCBs as an electron acceptor, with addition of the electron to the carbon-chlorine bond, chloride loss, and hydrogen abstraction from an unknown species.

concerted process catalyzed by this cobalamin cofactor or other corrinoids present in these anaerobic microorganisms.

D. Anaerobic Degradation

The dechlorination process described earlier does degrade highly chlorinated PCBs, but the organisms leave the biphenyl nucleus untouched and less chlorinated PCBs are formed. Although this dechlorination represents actual biodegradation of highly chlorinated PCBs, it is being distinguished here from processes that do attack the biphenyl ring, resulting in potential mineralization of the PCB. Such a process has recently been reported by Rhee and coworkers.[26,79] In this work, bacterial populations from Hudson River sediments were reported to anaerobically degrade the lightly chlorinated congeners in PCB mixtures. No metabolites were identified, and no evidence for the dechlorination process described earlier was observed by these authors. Although the dechlorination and biodegradation results reported here both utilized sediments from the Hudson River, CO_2 was provided to the dechlorinating systems as bicarbonate, but it was absent in the biodegradation studies. It is interesting to speculate that CO_2 may be important in determining the type of anaerobic activity observed on PCBs. It is possible that in the absence of CO_2, a selection is imposed favoring organisms capable of degrading PCBs to obtain CO_2 and/or low molecular weight metabolites as electron acceptors.

E. Summary

This environmental dechlorination of PCBs has now been observed in a large number of contaminated anaerobic sediments. Sites include many locations in the Hudson River (New York), Silver Lake (Pittsfield, Massachusetts), New Bedford Harbor (Massachusetts), Escambia Bay (Pensacola, Florida), Woods Pond (Massachusetts), the Housatonic River (Connecticut), the Sheboygan River (Wisconsin), Waukegan Harbor (Illinois), and the Hoosic River (North Adams, Massachusetts).[13,18] The widespread occurrence of this natural process indicates that it is a general phenomenon.

These changes observed in PCB-contaminated anaerobic river sediments led to the proposed microbial reductive dechlorination of PCBs.[15-17] This process has now been confirmed in a number of laboratories with sediments from many distinct aquatic systems.[2-4,78,78a,89,93] Some of the most significant findings from current anaerobic dechlorination experiments follow. (1) Dechlorination has been observed in a large number of sediments and the process is widespread in the environment. (2) Although congener preferences are demonstrated, in general the organisms present in Hudson River sediments exhibit broad dechlorination activity on the more highly chlorinated PCBs. (3) These anaerobic microorganisms are capable of dechlorinating even the previously recalcitrant, highly chlorinated PCB congeners contained in Aroclor 1260. (4) All results to date involve primary cultures and pure PCB dechlorinating strains have not yet been isolated. (5) Dechlorination selectively removes *meta* and *para* chlorines, significantly reducing any toxicity associated with PCBs. (6) The less chlorinated congeners that are produced are known substrates for aerobic bacterial systems.

IV. CONCLUSIONS

This paper has focused on recent progress (since 1985) in the aerobic bacterial biodegradation of PCBs and the new anaerobic dechlorination process recently discovered. The large number of researchers cited have repeatedly demonstrated that PCBs, commonly believed to be indestructible, are degraded by a number of diverse microorganisms.

Two separate and complementary natural biological systems have been the focus of this report on the biodegradation of PCBs. Anaerobic bacteria, present in river and lake sediments, remove chlorine from even the most highly chlorinated PCBs. This process is relatively broad, attacking a large array of highly chlorinated PCBs. The resultant lightly chlorinated compounds are less toxic, and are known substrates for aerobic bacterial biodegradation. Such aerobes have been identified in nearly all PCB-contaminated areas and are widespread in the environment. The obvious complementarity of these biological processes leads to the combined treatment scheme shown in Figure 9 (only one PCB congener is shown as an illustration). Successful application of this sequential treatment may enable the bioremediation of nearly all types of PCB contamination.

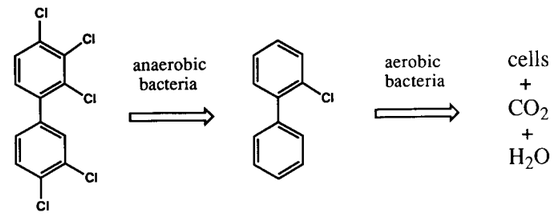

FIGURE 9. Two-step combined anaerobic/aerobic process to biodegrade PCBs. In this scheme, initial anaerobic treatment converts highly chlorinated PCBs to lightly chlorinated derivatives. Subsequent aerobic treatment destroys the remaining material.

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