Critical Review

Biological effects of Polychlorinated biphenyls and its bioremediation

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ABSTRACT
Polychlorinated biphenyls (PCB) are the most wide spread environmental pollutants and a prominent contaminant known to have toxic effects on humans and animals. They are well known as mutagens and carcinogens and affect almost all systems of the body. Due to their resistance to biodegradation and lipophilic properties, PCB bioaccumulate in tissues of human through regular food consumption. PCB are also known to cross the placenta and to be secreted into the mother’s milk, thus predisposing the infant to adverse health effects. Further, a higher incidence of bacterial infections was reported for breast-fed infants. While, data regarding the PCB-induced immuno toxic effects in humans are scarce, however data derived from experimental animals, include nonhuman primates, indicated that the immune system is a potential target for the immunotoxic effects of PCB. Additionally, PCB have the potential of partially antagonizing the effects of other structurally related compounds including the highly toxic dioxins, which are also present in small amounts in the environment. Thus, to fully evaluate the magnitude of the immunotoxic risk of PCB to humans, consideration should be given to investigate the interactive effects of PCB. In this context the present article focuses to reveal the biological effects of PCB and their bioremediation by bacteria.
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KEYWORDS
Polychlorinated biphenyls; Aerobic; Anaerobic bioremediation; Biodegradation.

INTRODUCTION
Polychlorinated biphenyls (PCB) are ubiquitous environmental contaminants that have attracted great concern because of their worldwide distribution, persistence in the environment, and possible deleterious effects. PCB were manufactured in the United States between 1929 and 1978 by Monsanto Corporation, and were marketed under the trade name Aroclor. Their electrical insulating properties and inflammability, combined with unique thermal and chemical stability, led to a wide variety of industrial uses as heat transfer fluids, hydraulic fluids, plasticizers, dielectric fluids, flame retardants, solvent extenders and organic diluents[1,2,15,70]. The widespread use of PCB coupled with improper disposal practices resulted in the discharge of large quantities of these environmental pollutants into non-target sites, such as soils, river and lake sediments and landfills. Thus, PCB have been routinely disposed of over years, without any precautions being taken. Serious environmental contamination with PCB has been documented for industrial areas such as the Great Lakes,
the Baltic Sea and Tokyo Bay. It is estimated that approximately 1.4 billion pounds of PCB have been manufactured and several million pounds have been released into the environment\(^2\). PCB are truly pervasive in the environment and would remain so far a long period of time, which was indicated by our laboratory experiments (Department of Microbiology, Davangere University, Davangere, India).

PCB are a class of chemical compounds in which chlorine atoms replace some or all of the hydrogen atoms on a biphenyl molecule (Figure 1).

![Polychlorinated Biphenyl (PCB)](image)

**Figure 1:** General chemical structure of PCB.

PCB are reported as contaminants in almost every component of the global ecosystem including air, water, soil, fish, wildlife, plants, domestic animals, human blood, adipose tissue and milk\(^{19,57,66}\). They can bioaccumulate in biological tissues, and their lipophilic behavior poses a serious threat to mammalian systems\(^{12,17}\). PCB are known to elicit a spectrum of toxic responses in humans, laboratory animals and wildlife including lethality, reproductive and developmental toxicity, body weight loss, dermal toxicity, liver damage, neurotoxicity, immunosuppressive effects, porphyria, teratogenic effects and carcinogenic effects\(^{45}\).

PCB can be biodegraded under both aerobic and anaerobic conditions. Degradation studies involving PCB have been largely conducted in estuarine and marine sediments. In general, much less effort has been given to the degradation of these contaminants in the soil environment. Also, toxic effects of PCB have been focused primarily on higher organisms and information on the toxicity of PCB on to the microbial ecosystem are scarce.

**BIOLOGICAL EFFECTS OF PCB**

The neurological effects of PCB have been extensively investigated in humans and animals. The main focus of most of these studies on the effects in neonates and young children, although few studies on adult have been conducted. One of the most important concern was the low level of PCB transferred to the fetus across the placenta would induce long-lasting neurological damage. Because PCB are lipophilic substances, there is also concern that significant amounts might be transferred to nursing infants via breast milk. These studies have provided evidence that PCB are important contributors to neurobehavioral alterations observed in newborn children such as motor immaturity and hyporeflexia. Some of these alterations even persist during childhood. There is preliminary evidence that highly chlorinated PCB congeners, which accumulate in certain fish, are associated with neuro-behavioral alterations seen in some newborn children. Children born to woman who accidentally consumed rice oil contaminated with relatively high amounts of PCB and Chlorinated Dibenzo Furans (CDFs) during pregnancy also had neuro-developmental changes\(^{25}\). Children exposed to PCB during fetal life showed IQ deficits, hyperactivity, and attention deficits, known as Autism\(^{13,40,52}\).

Additionally, PCB are found to cause liver cancer in the mice system\(^{53}\). The exact mechanism of hepatocarcinogenesis remained unclear. However, it was found that in female rats, and to a much lesser extent in male rats, there was pronounced iron accumulation in hepatocytes at the 26\(^{th}\) week when treated with mid- and high-dose of Aroclor-1254 and 1260. At 52 weeks, large accumulations of iron were also present in Kupffer cells of female rats, and dose-related increase in Proliferating Cell Nuclear Antigen hepatocyte labeling indices were found in both male and female rats. This study suggested that PCB-induced iron accumulation in hepatocytes was an early event, that would be related to tumor formation, especially in female rats\(^{67}\). Consequently, iron accumulations producing oxidative damage, and enhanced cell proliferation resulting in tumor promotion proposed to be components in the mode of action for PCB-induced hepatocarcinogenesis in rodents\(^{67}\).

PCB are considered potential endocrine disruptors due to their ability to act as estrogens, antiestrogens and goitrogens. Studies on the effect of PCB on sperm function and hormonal effects on rats revealed that tes-
tis weights were significantly increased whereas sperm count, motility, total motile sperm count, curvilinear velocity, average path velocity, straight-line velocity, and beat-cross frequency for motile sperm were significantly decreased. Further, there was a significant increase in thyroid-stimulating hormone level. However, no changes were seen in serum testosterone, thyroid hormones, or prolactin concentrations. These results suggested that the sperm functions may be more susceptible to endocrine disruption caused by dioxin-like PCB congeners.[33]

Additionally, endocrine disruption on environmental exposure to PCAHs (Polychlorinated Aromatic Hydrocarbons) would interfere with sexual maturation and in the long-run would adversely affect human reproduction.[32] Furthermore, exposure to PCB and their hydroxylated metabolites reduces fecundity and decreased circulating concentrations of thyroid hormones, resulting in serious reproductive and developmental defects. Thyroid hormones modulate both follicular development and steroidogenesis, and affect estrogen metabolism and the regulation of estrogen receptor[5].

The study on the effects of PCB mixture (Aroclor 1016) on follicle maturation in the Long-Evans hooded rat indicated that Aroclor significantly reduced the number of preantral follicles. T4 circumvented the Aroclor effect on the number of preantral follicles; however, a significant reduction in the antral follicle number persisted. In addition, a significant increase in atresia in the Aroclor treated ovaries were reported[5]. Study on the long-term exposure to PCB on developing dental enamel of 8 to 14-year-old children who were pre and postnatally exposed to PCB in the contaminated region of Bela Krajina, Slovenia revealed that the developmental defects of enamel in permanent teeth and demarcated opacities and hypoplasia. However, they reported that there was no significant correlations were found between PCB exposure and developmental defects in deciduous teeth[34].

Exposure to certain PCB can lead to development of cardiovascular diseases such as atherosclerosis. Although, very little is known about the mechanisms underlying this toxicity, endothelial cell dysfunction is a critical event in the initiation and acceleration of atherosclerosis. In one of the studies, porcine pulmonary artery-derived endothelial cells were exposed to three PCB congeners having different binding avidities with the Aryl hydrocarbon receptor, and differences in their induction of cytochrome P450 for up to 24 hours were recorded of the three PCB and two PCB, significantly disrupted the endothelial barrier function in a dose-dependent manner, by allowing an increase in albumin transfer across endothelial monolayer. These PCB also contributed markedly to cellular oxidative stress by 2,7-Di Chloro-Fluorescein and lipid hydro-peroxides, and caused a significant increase in intracellular calcium levels. Thus, certain PCB perhaps play a role in the development of atherosclerosis by causing endothelial cell dysfunction[59].

PCB have been found to alter the immune system in rodents, guinea pigs, rabbits and chicken as well as non-human primates[60,61] and indicated that higher chlorinated forms of PCB mixtures are more immunotoxic than the lower chlorinated Arochlors. Following exposure to PCB there is a reduction in the antibody production was also observed. However, there were variable changes with respect to thymus and spleen. There was significant reduction of thymus size in rats and rabbits[64] however, there was no change in mice system[55].

Alterations in the immune system have been observed in the Japanese and Taiwanese populations accidentally exposed to PCB through contaminated rice oil. There was significant effect on both cellular and humoral immunity[41,69]. As PCB can cross placenta and secreted through mothers milk severe adverse effect are found on the newborns[39,72]. Laboratory experiments exhibited reduction of antibody forming response to T-dependent antigen of sheep red blood cells, reduction of primary activation of T-cells by mixed lymphocyte response, and reduction of lymphocyte proliferation induced by various mitogens.

**PCB DEGRADATION BY BACTERIA**

Biodegradation by bacteria or other microorganisms, is a slow yet possible method for degrade PCB in both aerobic and anaerobic environments. It is the only process known to degrade PCB in soil systems or aquatic environments. The specific processes involved are aerobic oxidative dechlorination or hydrolytic dehalogenation and anaerobic reductive dechlorination. Theoretically, the biological degradation of PCB should
result to give CO₂, chlorine and water. This process involves the removal of chlorine from the biphenyl ring followed by cleavage and oxidation of the resulting compound. Persistence of PCB in the environment increases with the degree of chlorination of the congener. Those compounds with a high degree of chlorination are resistant to biodegradation and degrade slowly in the environment.

Aerobic Oxidative Dehalogenation involves the oxidation of PCB by aerobic microorganisms, especially by bacteria of the genus *Pseudomonas*. This involves the addition of oxygen to the biphenyl ring. It is also reported that the degradation of biphenyls was observed in *Micrococcus sp*. The metabolic pathway used by this family of bacteria resembles that described for the *Pseudomonas Sp*. By 1,2-dioxygenative ring cleavage, benzoate results as a common by-product of biphenyl degradation. Although different bacterial species seem to produce benzoate through PCB metabolism, further breakdown of benzoate seems to differ among the different microorganisms. Nevertheless, the by-products produced are less toxic compounds to living beings and the environment.

Since PCB are more persistent with increasing chlorination of the congener, aerobic biodegradation involving biphenyl ring cleavage, is restricted to the lightly chlorinated congeners. Anaerobic Reductive Dechlorination involves the replacement of chlorine with hydrogen atom on the biphenyl ring. This type of degradation transforms the more highly chlorinated congeners to less chlorinated ones. Specifically, the monochloro biphenyls and ortho substituted dichloro biphenyls are degraded in this manner. By products are less toxic and can usually be degraded by the aerobic microorganisms and the degradation process is found to be potential pathway for anaerobic degradation of a highly chlorinated congener to a less chlorinated one.

Reductive dechlorination of PCB has also been observed under methanogenic conditions. It has been suggested that the dechlorination of chloroaromatic compounds observed under methanogenic conditions, but inhibited under sulfate-reducing conditions, may be due to sulfate competing more effectively than the chloroaromatics for the electrons. On the other hand, anaerobic dehalogenation has been shown to take place in the presence of sulphate and degradation of chlorophenols would be coupled to sulphate reduction. In marine environments a wide variety of halogenated aliphatic and aromatic compounds are produced biologically by marine organisms. Therefore, anaerobic marine sediments may potentially allow for the selection and enrichment of anaerobic dehalogenating organisms.

It has been reported that an anaerobic bacteria, *Desulfomonile tiedjei* could dechlorinate 3-chlorobenzoate, was a energy (ATP) generating mechanism. Under conditions in the environment where electron acceptors are limiting, organisms with an ability to use PCB in this capacity may be selected for or enriched.

The different pathways of dechlorination observed may be explained by the different microbial populations that exist in the environment. However, a similarity between degradation patterns exists, in which the para- and meta-substituted congeners are more commonly degraded than ortho-substituted congeners. Only a few ortho-substituted congeners have been reported to degrade PCB. Moreover, anaerobic degradation has most commonly been observed under methanogenic conditions.

This would lead to conclude that anaerobic reductive dechlorination occur under methanogenic conditions, if not inhibited by sulfate-reducing conditions. Sulfates have a higher affinity for electrons than the chloroaromatics. In addition, many environmental factors can affect the degradation of biphenyls, both aerobically and anaerobically. Rates are quite variable depending on the conditions present in the environment. These factors may include; degree of chlorination, concentration on the congener, type of microbial population, available nutrients, pH and temperature and others.

As previously stated, more highly chlorinated congeners are less readily degraded than the less chlorinated congeners. The position of chlorine atoms on the rings also affects the rate of biodegradation. Not only are PCB with para- and meta-substituted rings more easily degraded than the ortho- substituted compounds, but PCB containing all chlorines on one ring are biodegraded faster than those which contain chlorines throughout both rings. It has been suggested that both aerobic and anaerobic conditions are affected with the addition
of certain nutrients. It is also interesting that biodegradation rates decrease with high levels of organic carbon being present\(^\text{[62]}\).

**BIOMETABOLIC PATHWAYS OF PCB DEGRADATION**

Aryl hydroxylating dioxygenases catalyze the addition of two hydroxyl groups to vicinal carbons of their substrates, thereby destroying the aromatic system and yielding dihydrodiol compounds of cis, cis stereochemistry\(^\text{[14,18]}\). The biphenyl dioxygenases (BphA) are members of this family of enzymes. The BphA have attracted attention as biocatalysts for the removal of polychlorinated biphenyls. Their use in this field was observed to be limited may be due to the typical pollutants consists of PCB mixtures of compounds, congeners.

Certain aerobic bacteria are able to oxidize some of the more highly substituted PCB congeners through a pathway that are basically identified in different organisms\(^\text{[3,8,27,42]}\). However, commercial PCB mixtures pose a huge problem to catabolic pathway as they typically consist of dozens of different congeners. So far, only a fraction of them can be attacked by known BphA. Therefore, enzymes with broadened and/or altered substrate ranges are in urgent need. These may be obtained either by more extensive and sophisticated screens of the natural resources\(^\text{[36]}\) or by the generation of novel substrate specificities through evolution in laboratory. Even if broad in the substrate range, no single pathway is able to metabolize all PCB in such mixture. Moreover, the characterized pathways convert a fraction of the PCB into dead-end metabolites\(^\text{[30]}\). Thus, enzymes with novel specificities that are useful to replace or supplement known ones are of particular interest.

The initial pathway enzyme, biphenyl dioxygenase (BphA), is of crucial importance for the successful breakdown of PCB. First, its substrate range frequently is narrower than that of subsequent pathway enzymes. Secondly, its regiospecificity of dioxygenation is a critical parameter, as it determines the potential sites of attack by the subsequent enzymes of the metabolic route. Thus, it controls further enzymatic degradation of a given congener.

During dechlorination pathway of PCB by anaerobic microorganisms replace the chlorine molecules with hydrogen atoms (reduction reaction), however, the final product would be still a biphenyl compound. Removal of chlorine atoms lead to increased water solubility and the rate of absorption thereby probably increasing the toxic effects. The end product acts as substrate for further metabolism by aerobic organisms\(^\text{[10]}\).

BphK is a glutathione-s-transferase of unclear physiological function that occurs in some bph pathways. It was demonstrated that BphK of *Burkholderia xenovorans* catalyzes the dehalogenation of 3-chloro 2-hydroxy-6-oxo-6-phenyl-2,4-dienoates (HOPDA) compounds that are produced by the cometabolism of PCB by the bph pathway and that inhibit the pathway’s hydrolase.

The PCB transforming capabilities of the bph pathway are strain dependent. Nevertheless, *Burkholderia xenovorans*\(^\text{[11]}\) poorly transform congeners containing more than four chlorine substituents. Therefore, it was clear by several observations that other bph enzymes are inhibited by specific chlorinated metabolites\(^\text{[21]}\). Additionally, the bphA1A2A3A4 gene cluster, encoding a biphenyl dioxygenase from *Rhodococcus globerulus* degrading a wide spectrum of PCB, was expressed in *Pseudomonas putida*, thereby allowing characterization of chlorobiphenyl oxidation by this enzyme\(^\text{[43]}\).

Chimeric enzymes have been investigated with the selection of chlorobiphenyls as potential substrate. This hybrid (BphA-B4h) harbours the core segment of a dioxygenase from *Pseudomonas sp*, isolated from a polluted sediment of the Elbe river, near Magdeburg, Germany\(^\text{[7]}\). The other sequences were provided by the BphA of *Burkholderia xenovorans*\(^\text{[29]}\) a metabolically very well characterized dioxygenase\(^\text{[54,73]}\). This showed that how substrate and product ranges of the hybrid enzymes differed from those of its parental BphA. Additionally, with several chlorobiphenyls, the newly generated deoxygenase showed complementing or improved degradative properties.

Nitrate reductase was also found to play an important role in the PCB degradation, as PCB were first degraded by reductive dechlorination. The ring cleavage probably would occur through production of non-specific peroxides by the white rot fungus. The importance of nitrate reductase enzyme on dechlorination has
been also documented by several research groups\cite{38,51,56}. It is also been documented that molybdenum which acts as cofactor for nitrate reductase increases PCB breakdown whereas tungsten which inhibits this enzyme decreases its breakdown.

**DETECTION OF PCB DEGRADATION**

Generally used method to detect degradation of PCB was Column gas chromatograms\cite{48,65}. If the Gas chromatograms of the environment PCB residues did not match with an appropriate cocktail containing known amounts of the commercial formulations of PCB, then, quantitation would be difficult. High-resolution isomeric specific PCB analysis is now a feasible option for the identification and quantification of the individual PCB present in commercial mixtures and environmental samples\cite{6,23,46}.

Thin-layer chromatography is a useful technique in pesticide residue analysis for the qualitative confirmation of results obtained by means of gas chromatography. While, an often used TLC- system like aluminum oxide/hexane was applied, however, very little information was obtained about the composition of the PCB residue. Only a diffuse and rather long-drawn spot was found with an approximate $R_f$-value between 0.6 and 0.8\cite{20}.

So far no TLC system was available that is particularly suitable for satisfactory separation of individual PCB compounds. Reversed-phase partition TLC (utilizing a non polar stationary phase and a polar mobile phase) has been used for resolving closely related lipophilic materials, such as homologous series of fatty acids\cite{20}.

Though the best method to study PCB degradation is by using GC/Mass Spectroscopy\cite{24,35}, The PCB breakdown products can also be studied by Nuclear Magnetic Resonance (NMR) or Infra-Red Spectroscopy (IR-Spectroscopy)\cite{50,63} where the different functional groups formed during the PCB degradation can be identified. High Pressure Liquid Chromatography (HPLC) has been used for a long time for the breakdown of PCB\cite{58}.

**CONCLUSION**

Polychlorinated biphenyls are one of the major recalcitrant having potential danger to the ecosystem. Although its production at large is banned, their effect in the soil sediments and transformer oil polluted area are prone to high risk. Therefore, there is a need to isolate the indigenous microorganisms and enhance their potential to degrade PCB more rapidly.

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**REFERENCES**

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