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Review

Biofilms at work: Bio-, phyto- and rhizoremediation approaches for soils contaminated with polychlorinated biphenyls

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Abstract: Organohalide contaminants such as polychlorinated biphenyls (PCBs) have been released into the environment for decades due to anthropogenic activities, but are also naturally produced in small amounts through volcanic eruptions and geochemical processes. Although toxic to humans and other organisms, the natural production of these compounds has resulted in the evolution of naturally occurring organohalide-respiring bacteria that possess the enzymes necessary to degrade PCB compounds to non-toxic products. The efficiency of PCB degradation can be improved by facilitating the formation of organohalide-respiring biofilms. During biofilm colonization on a surface or interface, bacteria are encased in an extracellular polymeric substance (EPS) or "slime," which allows them to share nutrients and remain protected from environmental stresses. Effective bioremediation of PCBs involves facilitation of biofilm growth to promote cooperation between bacteria, which can be further enhanced by the presence of certain plant species. This review aims to give an overview of biofilm processes involved in the detoxification of PCBs including anaerobic and aerobic PCB degradation by bacteria as well as the ability of plants to stimulate microbial activity and degradation (rhizoremediation and phytoremediation).

Keywords: polychlorinated biphenyls (PCBs); bioremediation; phytoremediation; rhizoremediation; anaerobic dechlorination; aerobic degradation; *Dehalobium chlorocoercia* (DF-1); *Burkholderia xenovorans* strain LB400; common tobacco (*Nicotiana tabacum*); switchgrass (*Panicum virgatum*)

1. Introduction

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that have toxic and carcinogenic characteristics. They were manufactured and used globally in industrial products such as coolants, paints and dielectric fluids until they were banned in the United States in 1979 and internationally in 2001 [1]. Commercially, PCBs were produced in the U.S. most commonly under the trade name Aroclor, which were mixtures of different congeners often found in oil emulsions [2]. The chemical configuration of PCBs consists of a biphenyl structure of two connected benzene rings, where some or all of the hydrogen atoms are substituted by chlorine atoms, resulting in 209 different congeners (Figure 1). The chemical formula is $C_{12}H_{10-n}Cl_n$, where the number of chlorines (n) ranges from 1 to 10 [3].

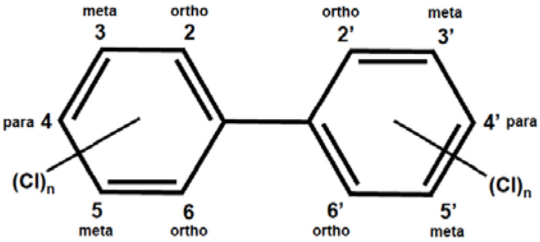

Figure 1. General chemical structure of PCBs.

The toxicity of a specific PCB congener depends on both the number of chlorine atoms and the chlorination pattern, which can lead to the structure of toxic, dioxin-like compounds [4]. PCBs were widely used in industrial processes due to their chemical stability, flame resistance and high evaporation point [3]. Their hydrophobicity enables adsorption onto soil and sediment particles, resulting in the bioaccumulation of the contaminant in organisms that live in or near PCB contaminated sites. Subsequently, biomagnification in the food chain occurs, since PCB breakdown due to natural biological processes is limited [5].

PCBs have been detected in many different environments such as landfills, wastewater biosolids, aquatic sediments, rivers, lakes and within the tissues of fish, turtles and birds living in contaminated areas [6,7]. In the U.S., 350 sites are listed by the Environmental Protection Agency (EPA) as having PCB contamination and have been placed on the National Priorities List (NPL) or "Superfund". The NPL is a list of critical sites threatened by the release of hazardous substances, pollutants or contaminants into the surrounding environment. Examples of sites include army and air force bases, industrial parks and water bodies such as the Great Lakes, Baltimore Harbor, New Bedford Harbor, Hudson River and Kalamazoo River [8–10]. In addition, PCBs have been detected in food sources and in human tissues where they can result in cancer, disruption of endocrine and immune systems as well as reduced reproduction [8].

PCB contamination is also an international issue, with measurable concentrations of PCBs found in the air in Germany, Yugoslavia, Japan and England [3]. In Norway and Sweden, human intake of PCBs has been studied and estimated at a daily toxic equivalent (TEQ) of up to 3.1 pg/kg of body weight, based on levels of dioxin-like PCBs found in blood [3,11]. In China, a study found PCBs throughout a municipal wastewater treatment plant that treats the discharge of an industrial producer of pigments and dies [12]. PCBs have also been observed in Antarctica, where they can be found in soil, sediment and ice cores. These cores can be analyzed and compared to recent industrial PCB emission data and historic volcanic eruption data to determine the anthropogenic and industrial sources of high concentrations of PCBs [13]. A 1996 study showed seawater, marine and lake sediment and soil samples from the Ross Sea and Victoria Land had typical PCB concentrations ranging from 130 to 240 (pg/g)/(m^2/cm^3) [14]. The global presence of large amounts of man-made PCBs found across a wide range of climate and terrestrial conditions calls for innovative methods to remediate these toxic chemicals.

This paper will review the influence of biofilms on PCB bioremediation, phytoremediation and rhizoremediation processes due to anaerobic and aerobic bacterial transformations by organohalide-respiring bacteria. In addition, aerobic uptake and transformation of lesser-chlorinated PCB congeners by plants and rhizospheric interaction between plants and bacteria will be considered. Finally, future research and development necessary in order to improve the efficiency of current field applications for remediation will be discussed.

2. Bioremediation Using Biofilms and Plants

Bioremediation to complete mineralization of PCBs requires two interrelated processes. The first process is anaerobic reductive dechlorination, where chlorine atoms are removed from highly chlorinated congeners using PCBs as electron acceptors. The second process is aerobic degradation, where the biphenyl ring structure is cleaved and mineralized using the PCBs as electron donors. This process leads to carbon dioxide and water as non-toxic breakdown products [15].

Bacterial biofilms are naturally occurring groups of microorganisms that colonize and accumulate on a surface or interface, which creates a unique niche different from that of planktonic bacteria [16]. Organohalide respiration, a recent term for the process by which halogens such as chlorine are transformed into a reduced form by bacterial respiration, can facilitate the anaerobic reductive dechlorination process [17]. Promoting the growth of beneficial biofilms on plant roots and soil particles enables on-site bioremediation rather than the traditional method of capping in-place or dredging contaminated soil or sediment and placing it in a landfill [6]. Successful bioremediation consists of identifying PCB dechlorinating and degrading bacterial species and their substrate needs in order to enhance transformation processes in the contaminated area. Supporting the colonization of strategically chosen bacteria in soil or sediment allows for faster, cheaper and more environmentally friendly cleanup of PCB contaminated sites [18].

Plants can also perform remediation in a process called phytoremediation. Certain plants located in contaminated areas can facilitate uptake and/or degradation of PCBs. Plants can also stimulate microbial activity due to their ability to provide an increased supply of organic carbon and catalytic enzymes. They are involved in numerous biological, chemical and physical processes, such as adsorption, accumulation, translocation and transformation, which can drastically affect the surrounding soil and aquatic environment [19,20]. Plant root zones are also active locations for microbial growth and activity. Rhizoremediation is a term that refers to the increased microbial degradation of a contaminant in a plant’s root zone (rhizosphere) due to the ability of contaminants to adsorb and bacteria to colonize and form biofilms on plant roots [21].

3. Hydrologic Life Cycle and Transformation of PCBs

When contamination occurs, PCBs are able to migrate throughout the environment and can often be detected large distances away from the source (Figure 2). Despite a production ban several decades ago, PCBs are still highly present in urban environments and are continuously released at low concentrations from urban and industrial activities (Step 1). When disposed of in a landfill or dumped in the environment, particles containing adsorbed PCBs can migrate into the surrounding soil and aquatic sediments (Step 2). Once they have entered the environment, PCBs are able to migrate into other municipal and industrial activities, such as water and wastewater treatment plants and agriculture. The major byproduct of wastewater treatment is residual organic material (biosolids), and some wastewater treatment plants struggle with PCB contamination throughout the plant and within biosolids samples. If contaminated biosolids are land-applied to fields, PCBs attached to the particles can re-enter the soils used for growing crops and are difficult to remove once integrated into site soil [7,21].

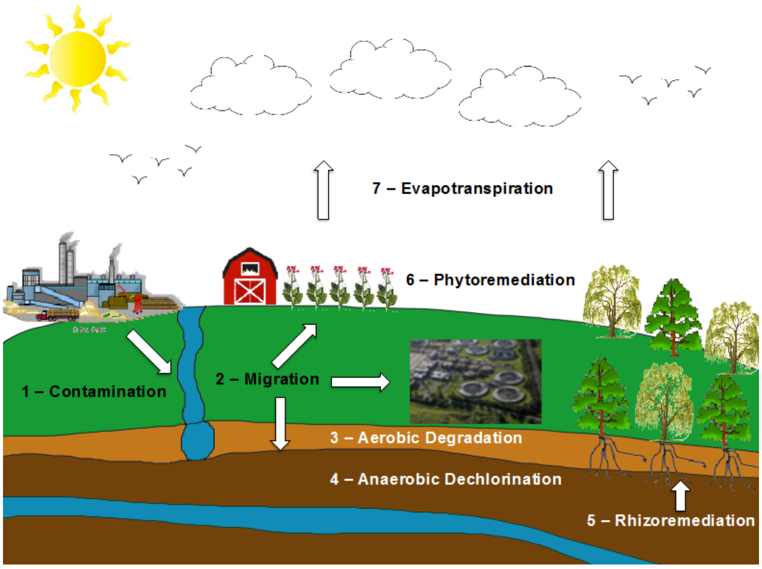

Figure 2. Processes involved in the hydrologic life cycle and transformation of PCBs in the environment.

Aerobic bacteria can be found in unsaturated environments often at or near the surface of soil and aquatic sediments (Step 3), whereas anaerobic bacteria are found a few centimeters below the surface in reduced, saturated zones (Step 4) [22]. Once the PCBs are stabilized and adsorbed to soil particles, anaerobic organohalide-respiring bacteria can form biofilms on particle and root surfaces in the contaminated area. These bacteria are capable of using PCBs as their electron acceptor to transform the original contaminant mixture into non-toxic products [15]. Both aerobic and anaerobic bacteria assist in the transformation and mineralization of PCBs in soils. In areas where plant roots are present, rhizoremediation can lead to the efficient transformation of PCBs (Step 5). In addition to transformation of PCBs by bacteria, some plants can also thrive in polluted soil and uptake PCBs into their tissue: a process known as phytoremediation (Step 6). Finally, if full PCB degradation is not achieved, transpiration by plants and evaporation of water from their leaves can transport the degradation products of PCBs into the atmosphere, where they can condense into clouds, precipitate and be re-deposited into the environment in various locations (Step 7, 23).

4. Anaerobic Bacterial Reductive Dechlorination of PCBs

Generally, PCB congeners with more than four chlorine atoms must undergo anaerobic microbial reductive dechlorination, or organohalide respiration, prior to aerobic degradation [21]. Through this anaerobic process, the number of chlorines per biphenyl molecule is reduced, thus creating PCB congeners with lower toxicity and improved aerobic biodegradability [22]. An example of a dehalorespiring bacterial isolate is *Dehalobium chlorocoercia* DF-1, from the class *Dehalococcoidia* of the *Chloroflexi* phylum [22,24,25]. This bacterium can dechlorinate double and single flanked PCBs at the *meta* and *para* positions, leaving chlorines in the *ortho* positions [15]. *Ortho* chlorines are important because they provide steric hindrance, and without the presence of *ortho* chlorines, toxic congeners with a co-planar shape are formed [26]. DF-1 removes *meta* and/or *para* chlorines forming less toxic, *ortho*-substituted chlorobiphenyls [27]. An example of this process is shown in Figure 3. Another strain, *Dehalococcoides mccartyi* CBDB1, is capable of transforming a wide variety of halogenated compounds such as biphenyls and dioxins [28]. Other dehalogenating strains include *Dehalococcoides ethenogenes* 195 and *o*-17 [29].

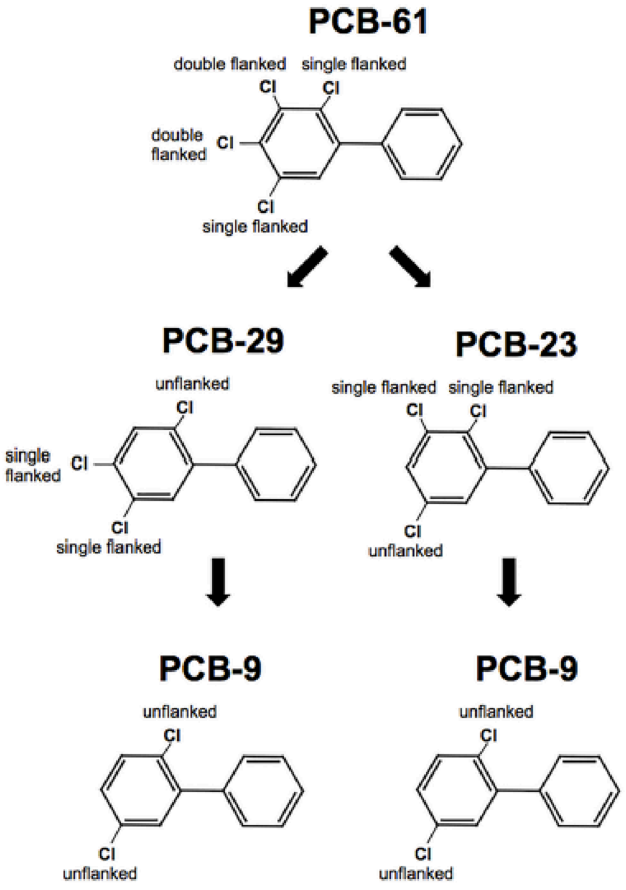

Figure 3. Anaerobic reductive dechlorination of PCB-61 to PCB-9.

5. Aerobic Bacterial Degradation of PCBs

Aerobic bacteria can usually degrade PCBs congeners with up to four chlorine atoms without prior anaerobic reductive dechlorination [30]. Both the number of chlorine atoms and their placement are key factors in the ability of bacteria to mineralize the molecule [24]. In the soil's unsaturated zone, aerobic bacteria inhabit the pore space of soil particles and form biofilms in order to cooperate and retain humidity [31]. Bacteria of the genera *Pseudomonas*, *Burkholderia, Comamonas*, *Ralstonia, Rhodococcus* and *Bacillus* have been shown to degrade PCBs aerobically, and most aerobic PCB degraders are Gram-positive [30]. Some of the most efficient aerobic PCB degraders are *Burkholderia xenovorans* strain LB400, *Rhodococcus sp.* strain RHA1, *Pseudomonas pseudoalcaligenes* strain KF707 and *Ralstonia eutropha*, which are capable of degrading a wide range of highly recalcitrant, congeners with up to five chlorine atoms [21,24,25,27]. Aerobic degradation of PCBs involves two clusters of *bph* genes, where the first cluster transforms the PCBs into chlorobenzoic acids, and the second gene cluster belongs to chlorobenzoate-degrading bacteria, which ultimately cleave the aromatic ring [27,30,32].

6. Aerobic Phytodegradation of PCBs

PCBs can enter plant tissues by diffusion through the cell membrane, which makes them available for degradation by plant genes. The PCBs are extracted from the soil, accumulate in the plant tissue and can be harvested and removed from the contaminated site. The uptake of PCBs by the plant depends mainly on the octanol-water partition coefficient [K_{OW}] of the congener mixture found on the site, but usually plants are only capable of translocating lower-chlorinated molecules with up to three chlorine atoms [21,33,34]. Certain plants, such as common tobacco (*Nicotiana tabacum),* common ivy (*Hedera helix*), cherry laurel (*Prunus laurocerasus*), common holly (*Ilex aquifolium*) and whole hybrid poplar (*Populus deltoides x nigra*, DN34) have an innate capability of degrading lesser-chlorinated PCB congeners [33,35,36]. Other food crops capable of PCB uptake are beet (*Beta vulgaris* L.), turnip (*Brassica rapa* L.), beans (*Phaseolus vulgaris*) and carrot (*Daucus carota*) [37,38].

Nicotiana tabacum is commercially cultivated worldwide for tobacco production for pipes and snuff. Because of its ability to grow in diverse environments and has shown enhanced PCB degradation potential compared to non-vegetated soil, tobacco is a good candidate for phytoremediation of PCBs [39]. In a study by Rezek et al. evaluating metabolites formed from PCBs in vitro by *Nicotiana tabacum*, researchers concluded that the PCB metabolites were maintained in the plant cells, demonstrating effective uptake of the PCB congeners via the tobacco cells [35].

In 2008, Moeckel et al. published an article on an experiment evaluating uptake kinetics and storage of PCBs in the cuticles and cuticle waxes from mature leaves of common ivy (*Hedera helix*), cherry laurel (*Prunus laurocerasus*) and common holly (*Ilex aquifolium*). The results showed that PCB uptake in waxes was higher on an area basis, however due to the large mass of cuticles compared to waxes, more PCBs could accumulate in cuticles than waxes. Generally, *Prunus laurocerasus* wax accumulated the most PCBs and *Hedera helix* wax accumulated the least PCBs per sample; however, *Hedera helix* had the highest PCB concentration per milligram of wax [36].

O'Connor et al. conducted an experiment to determine uptake of sludge-borne PCBs in carrot (*Daucus carota*). Despite degradation of PCBs in the soil, PCB contamination was only found in the peels of the carrots [38]. Sawhney and Hankin (1984) found that PCB uptake in beets (*Beta vulgaris* L.) and turnips (*Brassica rapa* L.) left a higher concentration of PCBs in leaves than roots [37]. Liu et al. determined that the stem of a woody, hybrid poplar plant accumulates more PCBs than the leaves [33]. Other known PCB-degrading plants include lettuce, tomato and Paul's Scarlet rose [40]. Although phytoremediation is a promising technology for the remediation of PCBs, proper disposal of contaminated plants must be regulated so PCBs are not consumed by humans and do not re-enter the environment once removed [41].

7. Importance of Biofilms in Rhizoremediation

Bioremediation of PCBs depends on factors such as the mixture of PCB congeners, the surrounding water movement and transport of molecules, the sediment composition, the presence of microorganisms and/or plants and the nutrients available to the microbial and plant populations [42]. Natural attenuation and transformation of PCBs will only occur with sufficient bioavailability of PCBs and abundance, diversity and activity of organohalide-respiring bacteria. Bacteria tend to favor the biofilm mode of growth due to increased stability and protection from environmental stresses when a cohesive bacterial community exists [43]. Plants have the capability to improve the degradation processes of microorganisms due to the ability of microbes form biofilms on the roots, reach greater soil depths and return nutrients to the plants to enhance growth [20]. As plant roots grow and eventually turnover, an oxygen and organic carbon supply is produced, and sugars, alcohols and organic acids are used as substrate by microorganisms [21]. Both the plants and bacteria are able to metabolize different PCB congeners, and by co-existing, full degradation of the contaminant can be reached [44].

Experiments have been conducted to determine which types of plants have a natural ability to thrive in PCB contaminated areas and stimulate the growth of PCB degrading bacteria. Pine trees (*Pinus* sp.), willow trees (*Salix* sp.), switchgrass (*Panicum virgatum*) and other tree and grass varieties have shown the ability to increase microbial PCB degradation in their root zones when compared to non-vegetated soil [25,33,45]. General results have shown an increase in degradation in vegetated soil compared to non-vegetated soil, as the rhizospheric interaction between plants and microbes has shown increased microbial activity and increased contaminant breakdown [21]. All PCB congeners have the ability to adsorb onto plant roots but due to compatibility with plant genes only lower chlorinated congeners are taken up into plant tissue and metabolized [24]. Therefore, it is important for plants and bacteria to cooperate to ensure full degradation of PCBs. *Rhodococci* are the primary PCB degraders associated with tree roots, due to their ability to use secondary plant compounds, such as phenolics, alkanoids and terpenoids, to degrade PCBs [25].

Liang et al. conducted experiments to determine the anaerobic dechlorinating potential of the microbial population associated with soil microcosms planted with switchgrass (*Panicum virgatum*). The results showed that PCB transformation products with few chlorine atoms were found in the microcosms, which indicated that dechlorination occurred in the soil environment. *Proteobacteria* and *Acidobacteria* are common bacteria found in soil and sediment and dominated the soil communities. Aerobic PCB degradation has been shown to occur by *Proteobacteria* such as *Pseudomonas, Sphingomonas, Acinetobacter, Comamonas and Burkholderia* [46]. Anaerobic dechlorinating *Chloroflexi* were not detected in the sample, possibly due to relative low abundance. However, PCB-118 was detected, and it requires *ortho* chlorine removal from PCB-153, which is performed by anaerobic bacteria. Although the abundance of organohalide-respiring bacteria was about 1% of total bacteria, their existence in the samples shows a promising dechlorination potential of switchgrass microbial communities [45].

8. Challenges and Future Research

There are many benefits to successful bioremediation, phytoremediation and rhizoremediation. Biofilm engineering is a beneficial approach that can be applied on-site, the major cost of transporting material can be offset and the primary energy source used is the sun [10]. However, there are some challenges that need to be addressed before bioremediation will become the preferred remediation technique. Firstly, the processes of plant and microbial transformation of PCBs are slow, and efficiency needs to be increased to have tangible outcomes within a shorter time period [21]. Plants and bacteria can also experience inactive and lag periods, where their success in PCB transformation can decline [30]. Bacterial populations are highly affected by variations in salinity, sulfate concentrations, temperature and pH, which can negatively impact remediation efforts [42]. Currently, there is a lack of knowledge on the identities of all PCB degrading bacteria, their abundance in the environment and how they form biofilms [47].

In the field, PCB contamination is usually widespread but at a low concentration, which makes the PCBs bioavailable to surrounding benthic organisms for consumption but unavailable for PCB degrading bacteria [21]. Recently, the addition of activated carbon as a material amendment has shown promise in reducing the bioavailability of PCBs in aquatic sediments and the food chain by adsorption onto the particles [10]. In addition, activated carbon provides a surface for suspended bacteria to attach to and form biofilms, which can increase the rate and efficiency of PCB degradation [48]. Bio-, phyto- and rhizoremediation approaches can also be improved by the use of transgenic plants and microbes, but regulations are necessary to ensure proper use of the transgenic organisms and their by-products [24].

9. Conclusion

Bioremediation of PCBs is an important scientific and engineering issue due to the persistence of the pollutant in the environment and the ability of the technology to decrease the cost of treating contaminated soil and sediment. Current technologies are limited due to the lack of knowledge on PCB dechlorinating and degrading bacteria and plants as well as the difficulty with identifying specific degradation pathways of PCB mixtures containing many different congeners. Furthermore, more research on identification of bacteria, rhizospheric interactions between plants and bacteria and *in situ* degradation scenarios is necessary to further the potential for bioremediation of PCBs to be a desirable clean-up method.

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Conflict of Interest

All authors declare no conflict of interest.

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