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Review

Mycoremediation of PCBs by *Pleurotus ostreatus*: Possibilities and Prospects

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Abstract: With the rising awareness on environmental issues and the increasing risks through industrial development, clean up remediation measures have become the need of the hour. Bioremediation has become increasingly popular owing to its environmentally friendly approaches and cost effectiveness. Polychlorinated biphenyls (PCBs) are an alarming threat to human welfare as well as the environment. They top the list of hazardous xenobiotics. The multiple effects these compounds render to the niche is not unassessed. Bioremediation does appear promising, with myco remediation having a clear edge over bacterial remediation. In the following review, the inputs of white-rot fungi in PCB remediation are examined and the lacunae in the practical application of this versatile technology highlighted. The unique abilities of *Pleurotus ostreatus* and its deliverables with respect to removal of PCBs are presented. The need for improvising *P. ostreatus*-mediated remediation is emphasized.

Keywords: mycoremediation; PCBs; Pleurotus; xenobiotics; fungus

1. Introduction

The synthetic compounds obtained through chlorination of biphenyls are called polychlorinated biphenyls (PCBs), which are composed of a biphenyl molecule (two benzene rings linked by a C–C bond) that carries one to ten chlorine atoms. PCBs as mixtures are commercialized with trade names Aroclor, Clophen, Delor, etc. These commercialized PCBs consist of a mixture of congeners distinguished based on the number and position of chlorines on the biphenyl nucleus. PCBs find place in numerous industrial applications, such as heat transfer fluids, dielectric fluids, hydraulic fluids, flame-retardants, solvent extenders, and organic diluents.

The use of PCBs is expanding and widespread these days, and these compounds are contributing more than enough damage to the environment with their percolation into soil and sedimenting, as there are inadequacies in their waste disposal [1,2]. According to a recent finding, traces of PCBs were still detected in places where production was carried on decades before, in spite of their restricted application then—and this does not even take into account the situation now with large-scale production, of late, to meet the large-scale applications. Currently, PCBs are considered as one of the most hazardous contaminants in the world, and hence, they are the topmost public health concern [3]. The teratogenic, carcinogenic, and endocrine-disrupting aspects of these xenobiotics have been well documented [4–9]. The most alarming adverse property of PCBs is their tendency toward bioaccumulation in lipid tissues and organic components of the soil and adipose tissue of animals and humans [10].

This review briefly dwells on the available bioremediation-based technologies for decontamination of PCBs with special focus on white-rot fungi and more so with *Pleurotus ostreatus*. The milestones achieved so far with *P. ostreatus* and their future prospects are presented.

2. Bioremediation of PCBs

With all the raising environmental considerations, the clean-up of PCB-contaminated sites has drawn everybody's attention and has become a top priority. Among the many remediation approaches that are available, the use of biological systems represents an effective, cost-competitive, and environmentally friendly alternative to the more commonly used thermal and physicochemical technologies [11]. The most prevalent existing practice in the removal of PCBs from contaminated materials is by incineration at high temperatures. In this procedure, the limitations to be faced are that it is expensive, brings additional risk of producing toxic chlorinated dioxins by combustion under lower temperatures, and has inherent volume limits. Therefore, studies on PCB biodegradation by microbes for the decontamination of water and soil systems have been gaining popularity. Decontamination of soil systems with respect to bioremediation has been well established compared to decontamination in water systems. With key advantages such as cost effectiveness and environmental friendliness gaining emphasis (as with any bioremediation system), bioremediation has received much acceptance.

Bacteria plays a key role in PCB biodegradation processes, it has been found. In addition, aerobes and anaerobes have also been reported to participate in their own way in the process. Highly chlorinated biphenyls can be used by anaerobes as electron acceptors, and it is possible to convert them into less chlorinated congeners. Aerobic microorganisms, on the other hand, can deal and co-metabolize lower chlorinated biphenyls [12–14]. Thus, both anaerobic–aerobic treatments are required to completely mineralize these xenobiotics. Bacterial PCB biodegradation in natural compartments is also well documented [15,16]. PCB congeners with four or more chlorine atoms undergo bacterial anaerobic reductive dechlorination, while the lower chlorinated PCB congeners are subjected to co-metabolic aerobic oxidation mediated by dioxygenases, encoded by the bphA gene family [17].

The process of PCB biodegradation mediated by fungi is also well established. Fungi's capacity to transform several PCB congeners in liquid medium have been described [18–36]. A few studies established the successful fungal transformation capacity in soils [18–24]. Unlike bacteria, ligninolytic cultures of *Phanerochaete chrysosporium*, a white-rot fungus, can mineralize tetrachloro- and hexachloro-substituted PCB congeners as well as Aroclor 1254 [37,38]. Other studies have proven that *P. chrysosporium* degrades higher levels (10 ppm) of Aroclor 1242, 1254, and 1260 [39].

Fungi, mostly wood-degrading basidiomycetes, are well established for PCB removal [40]. The elaborate fungal hyphae can easily penetrate into the polluted matrix. Additionally, the extracellular oxidative enzymes can scavenge even scarcely bioavailable contaminants by nonspecific radical-based reactions. Thus, comparing bacteria and fungi, the latter are said to be more acceptable and recognized for their inputs towards PCB removal. White-rot fungi (WRF) are the most active degraders of lignin to CO_2 in plants [41–44]. Earlier reports by Bumpus et al. [45] and Eaton [46] indicated that the white-rot fungus *P. chrysosporium* degrades dioxins, polychlorinated biphenyls (PCBs), and other chloroorganics. *P. chrysosporium* is the most extensively studied of the ligninolytic white-rot fungi that mineralizes xenobiotics [47–50]. Several white-rot fungi were tested for their ability to decompose PCBs [51]. Numerous studies have confirmed that white-rot fungi including *P. chrysosporium* [52], *Trametes versicolor* [53], *Lentinus edodes* [22], *Phlebia brevispora* [54], Irpex lacteus, Bjerkanderaadusta, *Pycnoporus cinnabarinus*, *Phanerochaete magnoliae* [55], and particularly *Pleurotus ostreatus* [28,55] could successfully orchestrate PCB removal [1]. However, only a relatively small number of white-rot species have been tested on real PCB-contaminated soil [29], although *P. ostreatus* is, thus far, likely the most efficient known PCB-degrading organism [28].

3. *P. ostreatus* Based Degradation of PCBs: Milestones Achieved

The oyster mushroom, *P. ostreatus*, is a common mushroom, first cultivated as a subsistence measure during World War I. Now grown commercially around the world for food, oyster mushrooms have been found more useful in industries, i.e., for mycoremediation purposes. As established by earlier researchers, *P. ostreatus* is the one that is now accepted to be highly promising for removal of PCBs. The state-of-the-art contribution of *P. ostreatus* in this area of bioremediation of PCBs is briefly presented below.

Zeddel et al., 1993 [26], demonstrated that *P. ostreatus* selectively removed PCBs from soil homogenized with wood chips. *P. ostreatus* was successfully applied at contaminant concentrations ranging from 100 to 650 ppm for single isomers and 2500 ppm for all PCBs. However, *P. chrysosporium* could not degrade any PCB except mono- and dichlorbiphenyl in a solid-state system under normal oxygen levels. However, with this limitation, *P. chrysosporium* was still reported to be the most versatile of the WRF to degrade Aroclor 1242, 1254, and 1260 [1]. However, with respect to bioremediation of PCB in soil, other WRFs like *Bjerkandera adusta*, *P. ostreatus*, and *T. versicolor* exhibited higher biodegradation than *P. chrysosporium.* Kubátová et al. [28], studied six strains of white-rot fungi for their biodegradation ability of low chlorinated polychlorinated biphenyl (PCB) in real soil system. *Phanerochaete chrysosporium* and *Trametes versicolor* did not show any ability to degrade PCBs in soil. By contrast, four strains of *P. ostreatus* were able to remove about 40% of Delor 103 in two months. All *P. ostreatus* strains decomposed PCBs selectively with the preference for congeners with chlorine atoms at ortho > meta > para positions. This study confirms *P. ostreatus*' unequivocal ability to perform in real world soil environments more efficiently compared to the others (who were all reported to be well accomplished laboratory scale achievers).

P. ostreatus produces ligninolytic enzymes which are able to interact with a plethora of waste substrates [33–35], including PCBs [36]. This mushroom's industrial cultivation faces a problem: the huge turnover of spent mushroom substrate (SMS), a lignocellulosic matrix, that has to be disposed of [56]. With this as the choke, the exploration of new applications for re-utilization of SMS became desirable. SMS is reported to contain high levels of residual extracellular oxidoreductases produced by the still metabolically active mycelium. It is the niche for active microbial community composed of fungi and bacteria [57,58]. Thus, application of SMS in bioremediation processes has been probed, and the ability of the SMS and its inherent microbiota to transform different contaminants has been reported [59–61]. SMS from *P. ostreatus* was previously validated as a low-cost organic substrate for remediation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil [62,63]. Moreover, lignosulfonate, an inducer of lignolytic activity, was found to possess no effect on the degradation of PCBs with *P. ostreatus* or *Trametes versicolor.* An oxygen concentration of 10% inside the substrate combined with 10% CO_2 also had no influence on the degradation potential of *P. ostreatus.* Monika et al. [64] carried out an experiment using a mixture of substrate/SMS and sandy soil with PCBs. The results indicated that degradation was dependent on substrate/SMS addition, the concentration of PCBs, and time of incubation. The degree of degradation of a single PCB after 12 weeks of incubation for *Agaricus bisporus* ranged from 31.32 ± 1.52 to 83.91 ± 1.07%, while for *P. ostreatus*, it was between 37.88 ± 2.54 and 78.29 ± 1.41% [65].

4. Mechanism of Bioremediation by *P. ostreatus*

It is known that extracts from WRF or their laccases catalyze the degradation of hydroxylated PCBs. However, as it stands, little is known about the in vivo mechanisms of PCB degradation [24,66]. Laccases degrade isolated PCBs congeners [67] or PCBs in commercial mixes such as Delor 103, Delor 106, and Arochlors 1242, 1254, and 1260 [1,58], but the limitation was that higher chlorination levels reduced the degradation efficiency. Most of these studies have been applied on low PCB concentrations only (1–2000 ppm). Canales et al., 2012 [36], determined the correlation of *P. ostreatus* laccase activity through their conformation on its transcript expression with the removal of high concentrations

(7100 ppm) of PCBs from Arochlor 1242 in liquid culture. This ability of *P. ostreatus* was significantly marked in that it was not influenced by the chlorination levels.

P. ostreatus, in the presence of a fungal growth substrate (e.g., lignocellulosic matrices), could transform PCBs in spiked and actually contaminated soils [26,27]. Myco-augmentation of contaminated matrices by this fungus is operational via substrate-unspecific extracellular and intracellular oxido reductases, laccases, and Mn-dependent and -independent peroxidases [28–30], enabling them to transform PCBs [24]. Additionally, *P. ostreatus* produces ligninolytic enzymes, which is an added asset, whereby it orchestrates the transformation of a plethora of waste substrates [31–33] including PCBs [34–36]. The exact mechanism whereby *P. ostreatus* degrades PCB is not worked out, but with the rich reservoirs of enzymes such as Lignin Peroxidases, managanese peroxidases, and laccases, there is no doubt that these enzymes will play a synergistic role, if not for individual contributions (Figure 1).

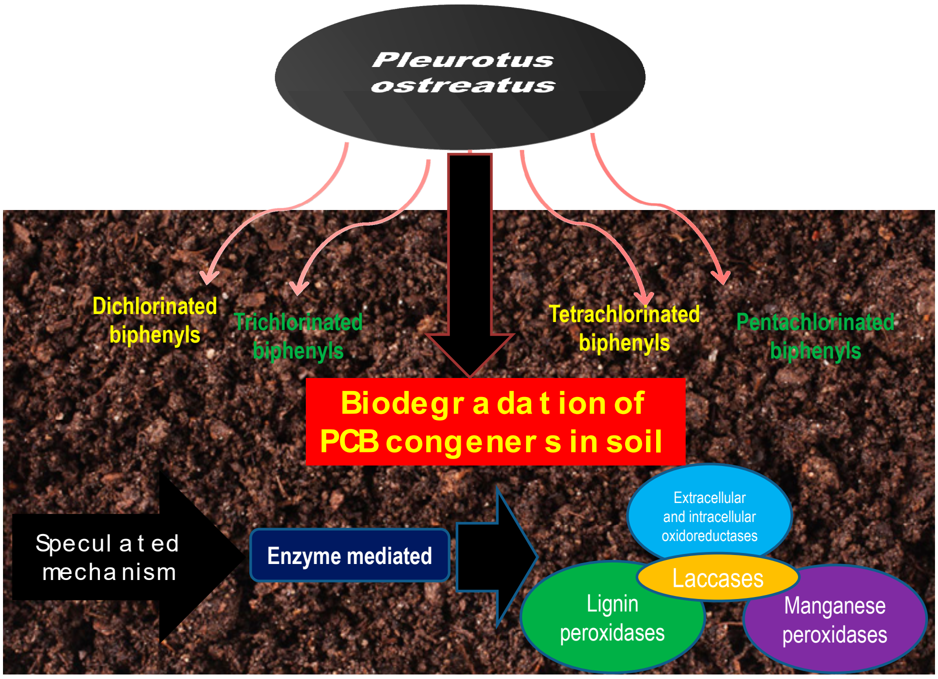

Figure 1. Schematic representation of the overview of bioremediation of polychlorinated biphenyls (PCBs) by *P. ostreatus*.

5. *P. ostreatus*-Based Degradation of PCBs: Future Prospects

As surmised in an earlier section, *P. ostreatus* does have an unequivocal edge over other WRFs when it comes to bioremediation of PCBs. The future prospects of this fungus towards the removal of PCBs are thus highly promising. In recent times, bioremediation processes more and more often employ immobilization methods. Immobilization is defined as limiting the mobility of the microbial cells or their enzymes with a simultaneous preservation of their viability and catalytic functions [68–72]. There are five main techniques of immobilization: adsorption, binding on a surface (electrostatic or covalent), flocculation (natural or artificial), entrapment, and encapsulation. This review identifies that no incorporation of such upgraded techniques available for preparing fungal masses for bioremediation protocols has been implanted for *P. ostreatus*-based degradation of PCBs. Since the fact that *P. ostreatus* can deliver much when it comes to PCBs remains unquestioned, it is now necessary that the biotechnological innovations are put to use for preparing this fungal biomass in the most efficient form. Most of the studies demonstrated on removal of PCBs using *P. ostreatus* merely use the hyphae as it is. More research on how the fungi can best be prepared for maximizing the PCB recovery is the need of the hour.

Immobilization of hyphae in columns as micro or nanobeads will enhance the surface-active area; further, incorporation of hyphae in polymer matrices as well as nanomaterials could significantly enhance the bioremediation aspects of *P. ostreatus* with respect to PCBs. Anna et al. recently presented an exhaustive list of options of natural carriers for bioremediation ranging from naturally occurring materials to nanopolymers [73]. With such options and advancements available, we strongly suggest some amount of improvisation into the routines to harness the best that this fungus has to offer toward bioremediation of PCBs. The SMS of *P. ostreatus* as explained in the previous section, which showed extensive PAH removal activity, is an area worth developing on with respect to PCBs. The SMS of *P. ostreatus* has been limited to a single study [64] for PCB degradation. This study showed that the differences between PCB degradation by substrate and SMS of *P. ostreatus* were very weak; hence, they suggested that it is possible to use SMS for the decontamination of PCB polluted soil. The Council Directive 1999/31/EC declared that each European Union country should reduce the amount of organic refuse by 50% by 2050 [74]. Poland, Korea, and China are the biggest producers of *P. ostreatus*, annually generating over a thousand tons of spent mushroom substrate. The proper channelizing of the SMS of this mushroom towards PCB remediation could solve the problem of disposal of this mushroom's refuse. Additionally, the use of the SMS allows for standardization of the exact dose requirement with respect to specific PCB concentrations or environmental conditions. Furthermore, SMS not only decontaminates the soil but is also a high value fertilizer. Further, every technology projection is weighed for its versatility and usefulness based on its cost effectiveness, with the SMS of this fungi showing degradation ability on par with the substrate/mycelium. In this direction, we are certainly talking of a major advantage in terms of cost-effectiveness while using the spent mushroom waste and not the mushroom itself. There is a high prospect that this venture could be highly prospective and cost-effective.

6. Conclusions

Only scattered reports and research are available in the direction of PCB degradation using *P. ostreatus*. This review hopes to enthuse researchers working on these lines to extend their biotechnological novelty aspects to this application, in order to harness the best that *P. ostreatus* could offer towards bioremediation of PCBs and other organopollutants. The utilization of SMS of this fungus, which would prove to be highly cost-effective, ideally needs to be entertained, and more real-time applications realized are emphasized.

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