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A comprehensive review on the application of mycoremediation in poly-chlorinated biphenyls treatment

Chelaliche Anibal Sebastian, Benitez Silvana Florencia, Alvarenga Adriana Elizabet, Zapata Pedro Dario, Fonseca Maria Isabel

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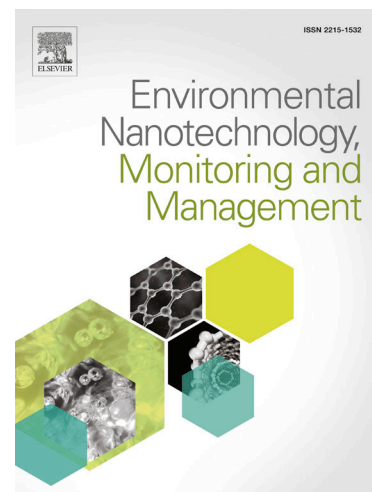
Title: A comprehensive review on the application of mycoremediation in polychlorinated biphenyls treatment

Author names and addresses: Chelaliche, Anibal Sebastian[12]; Benitez, Silvana Florencia[12]; Alvarenga, Adriana Elizabet[12]; Zapata, Pedro Dario[12]; Fonseca, Maria Isabel[12].

[1] *Universidad Nacional de Misiones. Facultad de Ciencias Exactas, Químicas y Naturales. Instituto de Biotecnología Misiones. Laboratorio de Biotecnología Molecular. CP3300 Posadas, Misiones, Argentina. Tel. +54 376 4480200. E-Mail: biotecmol2010@gmail.com*

[2] *CONICET. Buenos Aires, Argentina.*

Corresponding author: Chelaliche Anibal Sebastian. Laboratorio de Biotecnología Molecular, Instituto de Biotecnología Misiones "Dra. María Ebe Reca", Facultad de Ciencias Exactas Químicas y Naturales, UNaM, CP3300, Posadas, Misiones, Argentina. Tel. +54 376 4160192. e-Mail: sebastianchelaliche@conicet.gov.ar

Abstract

In the last decades, there has been a growing concern regarding the remediation and recovery of polychlorinated biphenyls (PCBs) contaminated sites. The technologies traditionally used are often energy-intensive, resource-heavy, and highly disruptive to the environments being treated. In this context, mycoremediation has emerged as a highly sought-after alternative due to the efficiency of certain fungal strains in achieving high removal percentages. This review provides an overview of mycoremediation strategies for PCB bioremediation. We begin by outlining the ecotoxicological challenges posed by PCB usage and traditional methods employed for remediating contaminated areas. Secondly, we present different approaches to mycoremediation of PCBs. The use of native PCB-degrading fungi shows that some strains belonging to the *Penicillium, Fusarium,* and *Scedosporium* genera are capable of removing over 70% of different PCBs congeners. Alternatively, we discuss using white rot fungi (WRF) due to their potential in transforming PCBs and associated metabolites. Strains belonging to this group, such as *Pleurotus pulmonarius*, can attain PCBs removal rates above 90% with a 10.27% reduction in toxicity. Additionally, cases demonstrating the application of WRF in long-term polluted soil and water are presented as field examples. A trickle bed pilot-scale bioreactor approach using *Pleurotus ostreatus* obtained an average PCBs removal of 89 ± 9% for contaminated groundwater. Similarly, microcosm experiments using *P. ostreatus* and *Irpex lacteus* removed up to 50.5% and 41.3% of PCBs content in long-term contaminated soils, respectively. We also highlight the role of extracellular ligninolytic enzymes, such as lacasses, lignin peroxidases, manganese peroxidase, manganese-independent peroxidase, and internal oxidoreductases in the PCBs metabolism carried out by WRF. Finally, we conclude with a series of factors to consider when implementing these techniques for remediating polluted sites, including up-scaling, current regulations, and combination with other remediation techniques.

Keywords

Introduction

Polychlorinated biphenyls (PCBs) are synthetic chemical compounds that consist of a biphenyl molecule with up to 10 chlorine atom substitutions, generating 209 possible congeners of PCBs (J. Liu et al., 2020), 60 of which are found in commercial products. The number and position of chlorine atoms on phenyl rings differentiate the PCB congeners (Reddy et al., 2019). PCBs can be categorized based on their structural similarity to polychlorinated dibenzo-p-dioxin (Pavuk et al., 2023). The dioxin-like PCBs' chlorine atoms are positioned in a non-ortho position, increasing the coplanarity of the molecule, whereas the non-dioxin PCBs present chlorine atoms in an ortho position, which decreases the coplanarity (J. Liu et al., 2020).

These compounds were produced and distributed worldwide between 1920-1970 as hydraulic fluids, lubricants, and equipment coolers due to their low combustibility, high chemical stability, and insulating properties (Biziuk & Beyer, 2020; Reddy et al., 2020). Also, PCBs have been used for heat transference, as plasticizers, dyes, and pigments (Anh et al., 2021). Approximately 1.5 million tons of legacy PCBs were produced during this period (J. Liu et al., 2020), and at least 30% of that production is distributed in the environment (Kandjo Ngoubeyou et al., 2022). In the past, a wide range of PCB-based products were produced under the names of *Aroclor* (USA), *Chlophen* (Germany), *Kaneclor* (Japan), and *Fenclor* (Italy) (Romianingsih, 2023). Although the production of these chemicals was concentrated in these countries, their transportation over long distances resulted in their distribution across continents, even in regions where their use was not certified (Reddy et al., 2019). PCBs have

been detected in air (Qu et al., 2019), water (Huang et al., 2020), soil (C. Liu et al., 2020), and sediments (Combi et al., 2020).

PCBs have been recognized as one of the most important persistent organic pollutants (POPs) since their incorporation into the priority list in the Stockholm Convention in 2001 (Lallas, 2001). Nowadays, PCBs are in the 5th position on the substance priority list of the Agency for Toxic Substances and Disease Registry (ATSDR), prioritized based on a combination of their frequency, toxicity, and potential for human exposure (ATSDR Priority List, 2022 - https://www.atsdr.cdc.gov/spl/index.html).

This review aims to describe the current knowledge of PCBs' mycoremediation approaches. We describe the impact and risk of PCBs in the environment and current remediation techniques and compare them to mycoremediation strategies regarding operational cost and effectiveness. We also highlight the different fungal strains involved in PCBs degradation, focusing the review on the action of WRF and their enzymes. Examples of field applications, as well as their advantages and limitations, are also discussed. Finally, we propose a series of considerations for future research regarding mycoremediation, such as limitations on the up-scaling from laboratory conditions, current regulations and guidelines for PCBs mycoremediation, and alternative approaches using genetic engineering.

Environmental impact and health risks

Despite being banned from production and use decades ago, PCBs persist in the environment due to their accumulation, low biodegradability, and ongoing accidental leakage (Kandjo Ngoubeyou et al., 2022). Though these pollutants are widespread in various environments, aquatic ecosystems (i.e., sediments, water, organisms) are considered their final repository. The discharge of PCB-contaminated wastewater is the main source of pollution; however, atmospheric deposition and washout from soils can also contribute to its presence (Kassegne et al., 2020; Reddy et al., 2019). When contaminated materials and equipment are disposed of improperly, rain and erosion can easily transport PCBs into nearby water bodies (Kandjo Ngoubeyou et al., 2022). Furthermore, contaminated air-borne particles can travel long distances and accumulate in remote environments. In this regard, PCBs have already been detected in zooplankton, fish, seals, and penguins from Antarctica in 1980-1990 (Larsson et al., 1992).

Due to their lipophilic nature, PCBs sorb onto organic matter and attach to suspended solids, sediments, and soil. They are also prone to bioaccumulate into fatty tissues, causing biomagnification across the food chain (Reddy et al., 2019). Lower trophic-level organisms are mostly contaminated through respiration and direct contact, and higher trophic-level organisms are mainly exposed through feeding. Environmental studies have shown that PCBs' concentration and distribution in organisms are directly proportional to lipid levels (Reddy et al., 2019; Klinčić et al., 2020; Kandjo Ngoubeyou et al., 2022). In aquatic ecosystems, biomagnification occurs from bottom-feeding species to large predatory animals; in terrestrial ecosystems, PCBs biomagnification progresses from soil/plants to insects, mammals, and birds. In both cases, humans are usually at the end of the biomagnification chain (Reddy et al., 2019).

PCBs can affect multiple animal systems and organs, causing poor development, malformations, and necrosis. They are endocrine disruptors, which can compromise the immune system, increase infection risk, and reduce the chance of survival. Bioaccumulation in tissues can also disrupt lipid metabolism, affecting energy production and leading to brain and reproductive problems. In fish, birds, and mammals, modifications in testicle morphology, egg and spermatozoid production, delayed puberty, infertility, neurological issues, abnormal

offspring, and several other detrimental effects were reported (Kandjo Ngoubeyou et al., 2022). In humans, PCBs were detected in multiple tissues: liver, kidneys, lungs, hair, blood, sperm, breastmilk, and even placenta (Zhu et al., 2022). PCBs exposure in humans has already been related to endocrine, reproductive, cardiovascular, neurological, and immune disorders (Montano et al., 2022). PCBs are also causal agents of malignant melanoma, and positive associations were found for non-Hodgkin lymphoma and breast cancer in human studies (IARC, 2016).

PCBs toxicity is strictly related to their structure due to the affinity of dioxin-like PCBs to the aryl hydrocarbon receptor (AhR). This transcription factor plays a key role in the metabolism of xenobiotics (Larigot et al., 2018) and the induction of oxidative stress, which produces cytotoxic effects. Damage occurs because of an oxidative disequilibrium coupled with the liberation of reactive oxygen species (ROS) during PCBs' metabolism (J. Liu et al., 2020). It has been proved that the Aroclor mixture 1254 and 1242 and PCB 153 increase ROS levels 1.8 times above normal physiological conditions (Mariussen et al., 2002). Additionally, PCBs are known for altering the antioxidant system that serves as a defense against oxidative stress (Perkins et al., 2016; Pessah et al., 2019) and are capable of producing a neurotoxic effect by altering the Ca^{2+} and neurotransmitter homeostasis (Rebuzzini et al., 2018; Westerink, 2014).

PCBs remediation methods

The presence, permanence, and toxicity of PCBs in the environment represent a serious ecotoxicological issue. The technologies available for the remediation of PCB-contaminated matrices are various, and their principal objective is to reduce concentration and mobility, given the matrix's physicochemical and biological characteristics (Alvarenga et al., 2018). These remediation methods can be divided into three categories according to the application site: *in situ*, *ex situ*, and dual remediation methods (Figure 1).

In situ methods

This type of remediation strategy is realized on the site to be treated without the movement or transportation of the soil or sediment (Sayqal & Ahmed, 2021). The most common methods within this category are:

- Natural attenuation: degradation occurs without intervention by microorganisms that naturally evolved in the polluted site (Terzaghi et al., 2020).

- Microwave Heating: this thermal remediation method effectively removes organic contaminants from soil and sediment in which the surface of the contaminated matrix is heated, breaking down xenobiotics (Krouzek et al., 2018).

- Capping involves depositing a layer of uncontaminated material (sediment or a combination of materials) over the contaminated sediments. This generally prevents the mobilization or resuspension of contaminants in the sediment and adjacent water bodies (Labianca et al., 2022).

- Decomposition by basic catalysis: applying a strong base and high temperature produces catalytic hydrogenation, removing chlorine atoms and replacing them with hydrogen atoms (Maghami et al., 2022; Liu et al., 2015).

Ex situ methods

These methods involve excavating and treating the soil or sediments before returning it to its original place (Sayqal & Ahmed, 2021). Among these strategies, the most commonly used for PCBs remediation are:

- Thermal desorption: volatile or semi-volatile contaminants in the soil are heated to an appropriate temperature by direct or indirect means under vacuum or using gas to separate the contaminant from the soil matrix (Zhao et al., 2019).

- Land farming: involves excavating the contaminated soil and transferring it to a prepared site, where the soil is tilled periodically until the contaminants are degraded by the microorganisms present in the same matrix. This method is generally limited to small portions of soil (Kaur et al., 2020).

- Excavation and landfilling: involves the excavation of the contaminated matrix and transportation to a suitable destination (landfill) to be treated afterward (Šrédlová et al., 2022).

Dual remediation methods

Some methods can be applied as both *in situ* and *ex situ* strategies depending on the needs and characteristics of the environment to be remediated. Among these are the following methods:

- Catalytic hydrodechlorination: transition metals are applied as catalysts and H_2 as a reducing agent in aqueous or organic solvents. For PCBs, it is an irreversible process in which chlorine atoms are removed from the molecule, resulting in a biphenyl molecule (Johnson & Lu 2024; Wu et al. 2012).

- Ferrous reduction: in this dechlorination method, the successive removal of chlorine atoms is carried out by a reducing agent, which in this case is zero-valent iron, producing the removal of chlorine atoms from the PCB molecule (Hashmi et al., 2022).

- Sodium dispersion: Sodium is dispersed in dry liquid ammonia under agitation, at room temperature, and in the presence of nitrogen. Under these conditions, the generated radicals can remove chlorine atoms from PCBs, forming biphenyls as end products (Fujimori et al., 2021).

- Photocatalytic dechlorination: a photocatalytic substance capable of absorbing light within the ultraviolet, visible, or infrared spectrum catalyzes the degradation of PCBs (such as titanium oxide photocatalytic systems) before extracting contaminants using surfactants (Hashmi et al., 2022).

- Ozonization: in this oxidative remediation strategy, ozone is used as a direct oxidizing agent (attacking the nucleophilic positions of organic substances and substituting functional groups of contaminants) or indirectly by forming hydroxyl radicals in the presence of water that decompose contaminant molecules (Joseph et al., 2021).

- Fenton reaction: during this strategy, the activation of hydrogen peroxide by iron salts produces hydroxyl radicals capable of degrading highly recalcitrant contaminants (Hashmi et al., 2022).

Bioremediation of PCBs

Most of the technologies mentioned above are often demanding from an energy and economic point of view, but they are also highly invasive and disruptive to the environment being remediated. In this context, bioremediation has been used to promote the degradation of these recalcitrant xenobiotics, with the additional advantage of having a lower environmental impact (Sadañoski et al., 2019). This strategy has received much attention because it represents an efficient biotechnological tool for environmental decontamination (Kour et al., 2021). There are also strategies such as biostimulation that accelerate the degradation of contaminants by microorganisms naturally inhabiting contaminated environments through optimization of factors such as nutrients, oxygen levels, temperature, and pH, among others. Some strategies enrich the soil with a microbial species or consortium capable of remediating the soil, also known as bioaugmentation (Nwankwegu et al., 2022).

Bioremediation given by microorganisms comprises two main degradation pathways: anaerobic and aerobic.

The anaerobic degradation usually occurs in PCB-contaminated sediments (freshwater lakes, rivers, and estuaries) and mostly involves the reduction of PCBs and the replacement of chlorine atoms with a hydrogen atom (Xiang et al., 2020). In their research, Bedard (1995) and Wiegel & Wu (2000) were the first to identify a series of dechlorination pathways in anaerobic bacteria. Through this type of microbial dechlorination, chlorines positioned at *para-* and *meta-*regions within the halogenated molecule are generally removed, primarily generating ortho-substituted congeners (Wiegel & Wu, 2000). Combinations of these pathways generate exclusively ortho-substituted congeners with a significant reduction in molecular planarity (J. Liu et al., 2020). However, this degradation pathway has the disadvantage of becoming less effective as the bioavailability and bioactivity decrease for highly chlorinated PCBs (Liu et al., 2007).

The aerobic biodegradation of PCBs involves an oxidative degradation that implies the breakdown of the biphenyl molecule, producing chlorobenzoic acid (CBA) and its subsequent degradation (Xiang et al., 2020). In bacteria, this process is carried out by the products of two gene clusters: one responsible for transforming PCBs into CBAs and the other responsible for further degradation. It has been documented that bacteria belonging to the genera *Pseudomonas*, *Ralstonia*, *Acinetobacter*, and *Rhodococcus* are capable of degrading PCBs through an aerobic metabolic pathway (Elangovan et al., 2019).

Bacterial degradation ability is given by an inverse proportion to the chlorination of the molecule; the more chlorine atoms present in the molecule, the more difficult it will be for bacterial metabolism to degrade PCBs (Wang et al., 2018). Moreover, most bacteria that degrade PCBs are not able to completely mineralize these compounds to carbon dioxide. This results in an accumulation of toxic intermediates such as benzoic acid and some varieties of CBAs, causing significant inhibitory effects on bacterial growth and consequently on the continuity of PCB degradation (Wojcik et al., 2020; Xing et al., 2020). An exception occurs with strain *Sphingobium fuliginis* HC3, capable of simultaneously degrading benzoic acid and 3-CBA (Hu et al., 2015).

Considerations for method selection

Given that the majority of the described methods can achieve high removal rates (Šrédlová et al., 2022), the choice for the remediation method depends mainly on the type and

concentration of contaminants present in the contaminated site, environmental impact, feasibility in terms of resources, available time, and dimensions of the contaminated area to be remediated. For each given situation, a careful consideration of the advantages and limitations of the methods must be accounted for.

For example, Sharov et al. (2019) reported the remediation of a highly contaminated site in Sumgait, Azerbaijan, with a PCBs concentration of 121.02 mg/kg, by excavation and landfilling. They first excavated 804 m^3 of contaminated soil and then disposed of the contaminated soil at the MENR Hazardous Waste Polygon (landfill). Using this approach, they achieved nearly a 97% removal rate of PCBs on the contaminated site. It has to be considered that since the contaminated soil was transported to a landfill, there was no actual remediation of contaminated material onsite in this case. However, due to the necessity of removing large volumes of highly contaminated soil and the disponibility of a hazardous waste storage facility, it was more practical to opt for this method. Other bioremediation approaches will take considerably more time to act on larger sites, and the tolerance towards high PCBs concentration must be considered a limitation to its applicability.

It is also important to note that in *ex situ* remediation methods, the transportation of the contaminated material may become a source of contamination for the environment and people. Kvasnicka et al. (2020) assessed the trade-offs of dredging as a PCBs remediation strategy. They monitored the dredging of the Hudson River Polychlorinated Biphenyls (PCBs) Superfund Site in New York, United States, between 2009 and 2015 (Kvasnicka et al., 2019). In this work, they compared the health benefits of reducing PCB levels in fish from the Hudson River against the health risks associated with increased inhalation exposures to PCBs and fine particulate matter derived from the transportation of dredged material. They concluded that dredging the Hudson River might have caused negative effects on human health, mostly due to fine particulate matter emissions generated during long- distance transport, which might exceed the reduced impact of ingesting PCBs in fish.

Other commonly used *ex situ* methods are incineration and thermal desorption. The incineration method is extremely efficient (above 99%) for treating small amounts of material with high PCBs concentration (Šrédlová et al., 2022). However, the cost of investment, operation, and land required to install the system is high (Adar et al., 2020; Trinh et al., 2019). The calculated cost for the incineration of hazardous liquid or solid wastes is about \$395-792/ton, considerably higher than other methods, such as advanced oxidation techniques (i.e., supercritical water oxidation), with an operating cost of \$76.56/ton (Adar et al., 2020). Another important disadvantage of incineration is the potential generation of toxic compounds (polychlorinated dioxins/furans), increasing the environmental impact (Trinh et al., 2019). On the other hand, thermal desorption is carried out at lower temperatures, decreasing the operation cost, but requiring the use of different types of additives (NaOH, $Ca(OH)_2$) and specialized filters for the generated gasses (Šrédlová et al., 2022). This method is also highly efficient, obtaining 94% of PCBs removal with 1% of $Ca(OH)_2$ at 600 $^\circ$C (Liu et al., 2019).

Ex situ approaches are useful when the site needs to be remediated quickly and it is possible to afford secure transportation to the specialized facility. Nonetheless, the underlying cost of transportation, operation, and collateral environmental impact make it difficult to apply them in some cases. Also, it must be noted that not all countries have access to facilities capable of carrying out these types of techniques and the economic resources to afford long-distance transportation (Adar et al., 2020). *In situ* remediation methods, on the other hand, can surpass some of these limitations while attaining high removal percentages.

In situ approaches are applied directly at the contaminated site, making it essential to assess environmental conditions to ensure effective implementation. For example, in capping strategies for sediment remediation utilizing activated carbon, highly dynamic conditions could result in a lesser efficiency due to sediment movement (Abel & Akkanen, 2018). Also, remediation

in environmental systems with ongoing contaminant influx may have lower efficacy for fish and other pelagic and epibenthic organisms (Gidley et al., 2019). However, when correctly used, activated carbon can greatly reduce the bioavailability of PCBs; a 90% reduction after 33 months was reported by Kirtay et al. (2018). Nonetheless, the toxic effects of activated carbon on organisms living in the sediment need to be contemplated, given that the smaller the particle, the higher its toxicity (Abel et al., 2017).

In situ bioremediation strategies usually offer a reduced environmental impact compared to other approaches (either *in situ* or *ex situ*). Some simple biostimulation approaches, such as adding pulverized pine needle biomass, can help decrease the PCBs concentration in soil by 38% (Lehtinen et al., 2014). Additionally, the change of oxygen conditions in sediment can shift the bacterial community composition towards potential degrading genera, achieving 32–58% degradation of CBAs (Matturro et al., 2020). Even though the degradation of CBAs could reduce toxicity, the ecological implications of this shift in the community composition in the natural environment must be further explored. Other bioremediation approaches may include incorporating species isolated from contaminated soil and later added to another contaminated site. Cervantes-González et al. (2019) isolated bacteria belonging to the genera *Achromobacter*, *Bacillus*, and *Pseudomonas* from PCBs contaminated sites and applied them while adjusting the carbon:nitrogen:phosphorus ratio. They reduced PCBs concentration in soil by 60% in a laboratory microcosm.

When comparing PCBs bioaugmentation or biostimulation techniques to other more classical approaches, it can be noted that the percentages of removal are usually lower. In this context, fungal remediation strategies may help get degradation rates closer to those of classical methods. Fungi represent the most prospective organisms in degradation and remediation, and their inclusion in bioremediation studies has been largely applied, from laboratory-controlled conditions to pilot-scale remediation strategies of environmental matrices historically contaminated with PCBs (Šrédlová et al., 2022).

Understanding the Versatility of Fungal Biodegradation

The use of fungi in remediation strategies is known as mycoremediation. Mycoremediation is considered an economical, eco-friendly, and effective strategy that takes advantage of this taxon's robust hyphal growth. Also, the production of extracellular enzymes, large surface area, and adaptability make them a promising group for remediating contaminated sediments and soils (Akhtar & Mannan, 2020).

Unlike bacteria, the movement of fungi through the contaminated matrix is not affected by the heterogeneity and complexity of the pores in said matrix nor by hydrological conditions dynamics (Espinosa-Ortiz et al., 2022). The production of hydrophobins allows hyphae to break the air-liquid interface, favoring fungal growth within unsaturated pores in the matrix (Antón-Herrero et al., 2023), which would allow better access to contaminants, a limiting condition in case of microbial degradation (Espinosa-Ortiz et al., 2022). In this sense, the presence of filamentous fungi can enable the dispersion of both contaminants (Moeder et al., 2005) and degrading bacteria (Kohlmeier et al., 2005; Worrich et al., 2016), thus functioning as "hyphal highways".

Fungal metabolic adaptations at different biological levels in response to contaminants may vary among species. Some fungal species indigenous to contaminated sites could turn promising for bioremediation processes. These strains are naturally selected by their environment and can develop in the presence of the xenobiotic (Marco-Urrea et al., 2015). As for PCBs, species belonging to the genus *Fusarium, Thermothelomyces, Thermoascus,* and *Schizophyllum* isolated from contaminated sites showed a higher tolerance to PCBs compared to the same species isolated from non-contaminated sites (Périgon et al., 2019). Furthermore, PCBs-contaminated isolates identified as *Aspergillus, Penicillium, Scedosporium, Phoma,* and *Doratomyces* species have shown tolerance and PCBs' removal capacities (Mouhamadou

et al., 2013; Tigini et al., 2009). The involved enzymes in the degradation of PCBs by these native strains have not been deeply studied. Germain et al. (2021) evaluated the extracellular enzymes of several native fungal strains adapted to PCBs. In this work, they only found significant laccase activity in a *Trametes versicolor* strain but no other significant extracellular enzymatic activity. It is suggested that the degradation of PCBs by these strains could involve intracellular enzymatic systems (Harms et al., 2011).

Even though fungi are able to adapt to contaminants, a subgroup within filamentous fungi, called "white-rot fungi" (WRF), has demonstrated PCBs-degrading capabilities without being previously exposed to them. These fungi, mostly from the Basidiomycota division, are considered a physiological group capable of degrading lignin and similar compounds, leaving a whitish coloration on the decaying wood (Bilal et al., 2017). This group of fungi can degrade lignin and cellulose biopolymers by producing a battery of enzymes with synergistic action (Suryadi et al., 2022). These extracellular oxidative enzymes participating in the ligninolytic system allow them to transform and degrade environmental contaminants due to their low substrate specificity. WRF belonging to the *Phlebia, Irpex, Pleurotus,* and *Pycnoporus* genera isolated from natural environments have shown tolerance against PCBs as well as a significative production of ligninolytic enzymes, such as laccases, manganese peroxidase, and lignin peroxidase (Sadañoski et al., 2018).

The ligninolytic system of WRF is mostly composed of extracellular enzymes such as decolorizing peroxidases (EC 1.11.1.19, DyP), lignin peroxidases (EC 1.11.1.14, LiP), manganese peroxidases (EC 1.11.1.13, MnP), versatile peroxidases (EC 1.11.1.16, VP) and laccases (1.10.3.2, Lac) (Zhuo & Fan, 2021). This extracellular system represents a powerful defense mechanism against xenobiotic compounds and is differentially distributed within WRF (Bilal et al., 2017). According to their enzyme production, WRF can be grouped into:

- Strains able to produce LiP, MnP, and Lac.

- Strains able to produce MnP and Lac.

- Strains able to produce LiP and MnP

- Strains able to produce LiP and Lac.

The previously mentioned enzymes attack these organic compounds through a wide range of redox reactions, often involving ROS capable of degrading xenobiotics into byproducts that can be utilized by the fungal metabolism itself, even leading to the mineralization of contaminants (Korcan et al., 2013).

Some specific extracellular enzymes have shown a degradation potential that makes them excellent candidates for application in bioremediation. Among these enzymes, we can mention:

- Manganese peroxidase: is an enzyme that contains a heme group, first discovered in the fungus *Phanerochaete chrysosporium*, and is described as the most common lignin-modifying peroxidase of WRF (Zhuo & Fan, 2021). The catalytic activity of MnP depends on the manganese ion, preferring Mn^{+2}, which can be oxidized to Mn^{+3}. Chelated Mn^{+3} can serve as a mediator for charge transfer, allowing the oxidation of phenolic substrates such as simple phenols and dyes (Manavalan et al., 2015). This enzyme can oxidize and degrade non-phenolic structures with the assistance of lipid radicals (Falade et al., 2017).

- Lignin peroxidase: this enzyme was first reported in *P. chrysosporium*, and is classified within the oxidoreductase peroxidase group. It has a high redox potential, giving it an advantage in oxidizing non-phenolic structures (Cui et al., 2022). In addition, it can oxidize aromatic compounds and organic contaminants (Lothe et al., 2020). This enzyme is related to a common secondary metabolite in WRF, veratryl alcohol, which is capable of moderating the inactivation of LiP by hydrogen peroxide. This metabolite can be used in strategies that include this enzyme to accelerate the transformation of contaminants (Singh et al., 2021).

- Versatile peroxidase: this enzyme is a ligninolytic peroxidase that contains a heme group combining the properties of MnP and LiP (Manavalan et al., 2015). They can oxidize phenolic substrates by oxidizing Mn^{+2} to Mn^{+3} and non-phenolic aromatic compounds similarly to LiP (Falade et al., 2017). This versatility allows its application in reactions involving both high and low redox potential, being useful for catalyzing a wider range of organic pollutants.

- Dye-decolorizing peroxidases: are enzymes named for their ability to decolorize compounds, namely dyes. They are widely distributed among Agaromycetes, other fungi, and bacteria (Colpa et al., 2014). DyPs share a particular protein structure and amino acid sequence and show a preference for anthraquinone dyes. They have high peroxidase activity against various organic compounds. It has been observed that they can exhibit oxidase activity without hydrogen peroxide (Lauber et al., 2017).

- Laccases: oxidases that contain several copper ions that catalyze single-electron reactions with the reduction of molecular oxygen to water. These enzymes are widely distributed among WRF. Their non-specific oxidation and use of oxygen as an electron acceptor make them candidates for various industrial and environmental applications (Zhuo et al., 2019). Lac produced by WRF are some of the most widely studied enzymes for bioremediation, combined with strategies such as immobilization on various substrates and combination with advanced oxidation processes (Zhuo & Fan, 2021).

- Cytochrome P450: this is the only intracellularly expressed enzyme. These heme group-carrying enzymes are one of the largest protein families, are present in all living organisms (Shin et al., 2018), and are known for their detoxifying ability. These enzymes act as terminal monooxygenases that catalyze hydroxylations, heteroatom oxidations, dealkylations, epoxidations, reductions, and dehalogenations (Bernhardt, 2006). Generally, eukaryotic cytochrome P450 systems have a P450 monooxygenase and a P450 oxidoreductase associated with the membrane (Syed et al., 2010). The P450 monooxygenases from WRF have been applied to remove and detoxify a wide range of xenobiotics.

Figure 2 shows some advantages of working with WRF in mycoremediation strategies.

Fungal Species and PCB Transformation

Reports of PCB-degrading fungi began in the 1980's. The first strains described to be able to mineralize these compounds were *F. oxysporum* (Sansur, 1982) and *P. crhysosporium* (Eaton, 1985); several more have been studied since (Kaleem et al., 2023).

Mycoremediation strategies usually involve degrading strains screening that can be native or exogenous to the contaminated matrix. In this regard, researchers have isolated,

characterized, and evaluated fungal strains from long-term PCB-contaminated soils (Germain et al., 2021; Mouhamadou et al., 2013; Tigini et al., 2009), i.e., strains acclimated to the pollutant. However, strains isolated from contaminated sites are not necessarily tolerant (Tigini et al., 2009), and tolerance assessment is often the first step in the search for degrading strains. Fungal tolerance can be studied in solid or liquid media. In the case of solid media, Bevilacqua et al. (2017) proposed fungal growth modeling in the presence of the contaminant as a simple method for strain selection. This method was also applied for WRF by Sadañoski et al. (2018), who evaluated growth and lignonolytic enzyme production using predictive mycology as a tool for promising PCB-degrading strain selection.

Most of the native PCB-degrading fungi belong to the Ascomycota division. Tigini et al. (2009) isolated 21 strains from contaminated soil, five of which were able to degrade the pollutant in liquid media, and *Penicillium chrysogenum* demonstrated notable efficacy with a degradation rate of over 70%. In a similar study, Germain et al. (2021) isolated 12 strains, four of which were capable of PCBs. In this work, *P. chrysogenum* achieved over 95% removal; however, increased toxicity was determined after treatment. Furthermore, *Penicillium canescens* was the only strain capable of reducing toxicity, although with a lower removal rate. It is well-established that toxic metabolites can be generated during PCBs treatment (Liu et al., 2022), encouraging researchers to study other taxa that could metabolize PCBs and their degradation products.

As previously stated, WRF have a versatile metabolism and high tolerance towards pollutants (Patel et al., 2020). Sadañoski et al. (2018) evaluated the tolerance of 26 strains isolated from a subtropical rainforest. Most of the strains were able to grow in the presence of PCBs, and some of them tolerated high concentrations of the pollutant. Several strains were probed to have PCB-degrading abilities (Chandra et al., 2021), some examples include *P. crhysosporium* (Eaton, 1985)*, T. versicolor* (Zeddel et al., 1993; Köller et al., 2000)*, Lentinus edodes* (Ruiz-Aguilar et al., 2002)*, Pleurotus ostreatus* (Zeddel et al., 1993; Čvančarová et al., 2012*, Šrédlová et al., 2020)*, Phlebia brevispora* (Kamei, Sonoki, et al., 2006)*, Trametes sanguinea* (Benitez et al., 2021) and *Pleurotus pulmonarius* (Chelaliche et al., 2021; Sadañoski, Benitez, et al., 2020). *P. chrysosporium* is known for its role in mycoremediation, and pollutant degradation by this fungus has been thoroughly evaluated (Kaleem et al., 2023). However, the genera *Pleurotus* has also shown a remarkable ability to remove PCBs in different matrixes. *P. ostreatus* selectively removed PCBs in soil homogenized with wood chips (Zeddel et al., 1993) and reduced 40% of the concentration of Delor 103 in a real soil system (Kubátová et al., 2001). In liquid media, *P. ostreatus* (Čvančarová et al., 2012; Moeder et al., 2005) showed promising results during PCBs degradation. Within this group, the strain *P. pulmonarius* LBM 105 (previously identified as *Pleurotus sajor-caju*) was able to degrade a mixture of Aroclors 1242, 1254, and 1260 in liquid media (Chelaliche et al., 2021; Sadañoski et al., 2019). This fungus can achieve degradation percentages above 90% in liquid media after 24 days (Benitez et al., 2021). Moreover, both fungal species were capable of reducing toxicity, although this was influenced by the treatment conditions (Sadañoski et al., 2019; Šrédlová et al., 2020).

Several studies showed that degradation and detoxification rates are influenced by the culture conditions, fungal strain, presence of other organisms, PCBs concentration, chlorination degree, and substitution pattern. Mouhamadou et al. (2013) and Germain et al. (2021) reported that the removal rate for fungi isolated from contaminated soils was not affected by the degree of chlorination; however, the removal was different among species and strains of the same species. In the case of WRF, Kamei, Kogura et al. (2006) determined that the removal rate for *P. crhysosporium* was higher in a low-nitrogen, high-carbon liquid media, while the strain *Phanerochaete* sp. MZ142 performed better in potato dextrose broth. They also determined that the degradation rate was affected by the substitution pattern and not the chlorination degree. Sadañoski, Benitez et al. (2020) reported higher degradation rates in a mineral nitrogen-limited media, compared to a complex media, for *Irpex lacteus* and *T.*

sanguinea; conversely, this was not the case for *P. pulmonarius* (Sadañoski et al., 2019). Despite the variability of these results, it is pointed out that fungi require a carbon source to grow and co-metabolize PCBs (Tigini et al., 2009; Stella et al., 2017).

PCBs with low chlorine substitution are usually more easily degraded by fungal metabolism. When degrading a complex Aroclor mixture, it was evidenced that *P. pulmonarius* LBM 105 degraded a higher fraction of Aroclor 1242, which proportionally contains a high quantity of PCBs congeners with a lower chlorine content. The percentages of removal studied on the same day showed a slight diminution in the capacity of this strain to remove Aroclors 1254 and 1260, which are characterized by a higher chlorine content (Sadañoski et al., 2019). From the degradation study of Delor 103 by *P. ostreatus*, Čvančarová et al. (2012) determined that in almost every PCBs concentration assayed, this strain was able to degrade tri- and tetrachlorinated PCBs, while the degradation of congeners with higher chlorine content varied depending on the total concentration of PCBs. Similarly, the PCBs mineralization by WRF tends to decrease with an increase in the chlorine content, with percentages of mineralization ranging from 11-16% for mono-, di- and trichlorinated biphenyls, while 0.4-1.4% mineralization was observed for tetra- and hexachlorinated biphenyls (Chandra et al., 2021).

The previous examples correspond to axenic cultures, which are important for establishing potential degrading strains and understanding metabolic pathways. However, evaluating PCBs degradation in non-sterile conditions is crucial since it is closer to real conditions. Šrédlová et al. (2020) studied the degradation of PCBs in contaminated water treated with *P. ostreatus*, concluding that the strain can grow in the presence of other organisms and that the treatment was more effective for low-chlorinated PCBs. As for solid matrixes, Ruiz-Aguilar et al. (2002) determined that the interaction between *P. chrysosporium* and the indigenous microorganisms in contaminated soil promoted the degradation of PCBs. Similar results were reported by Stella et al. (2017) for contaminated soils treated with *P. ostreatus*, where stimulation of the native bacteria was observed. The main challenge of applying WRF to soils is to ensure its growth and viability. Several authors have established that lignocellulosic wastes can be used for fungal immobilization or as amendments to overcome this (Ruiz-Aguilar et al., 2002; Sadañoski, Tatarin et al., 2020).

The effects of different parameters on bioremediation strategies are key points to be considered to achieve high removal efficiency (Rahman et al., 2023; Malik et al., 2020). In addition to nutrients, other factors that influence mycoremediation effectiveness are pH, temperature, oxygen levels, and moisture content (Dickson et al., 2019). In soil treatment, moisture levels are typically maintained above 60% to favor fungal growth and degradation capacity (Akpasi et al., 2023). For organic pollutants, higher temperatures usually increase solubility, bioavailability, and degradation rates (Dickson et al., 2019; Akpasi et al., 2023). As for pH, the degradation of organic compounds in soils was reported to be higher in neutral to alkaline conditions (Dickson et al., 2019), affecting their solubility, precipitation, and availability (Akpasi et al., 2023). Both temperature and pH also impact fungal growth, enzyme activities, and binding affinity, which varies depending on the applied strain (Akpasi et al., 2023). In the specific case of PCBs mycoremediation, reports on the effect of these factors on degradation/detoxification rates were not found in the existing literature.

Interestingly, the interaction between fungi and native bacteria could result in better overall remediation of the contaminated site since fungi increase the bioavailability or reduce the harmful effects of these toxic compounds by conversion to soluble intermediates, and bacteria would use these intermediates as substrates and perform further mineralization (Bokade & Bajaj, 2023). For polycyclic aromatic hydrocarbons (PAHs), fungi can mobilize sequestered compounds by accumulation on their vesicles, making them available for uptake (Bokade & Bajaj, 2023). Bacteria can contribute to fungal development by the production of fungal growth-promoting metabolites (Tunsagool et al., 2023), including indole acetic acid (IAA) and phosphate (Dou et al., 2024). The use of spent mushroom substrate (SMS) of *P.*

ostreatus showed an increase in the quantity of metabolic active bacteria *(Acidobacteria, Firmicutes,* α and β *Proteobacteria,* and *Bacteroidetes*), and an increase in Lac activity and PCBs depletion over time (Siracusa et al., 2017)*.* In microcosm experiments the presence of *P. ostreatus* appeared to stimulate the growth of bacteria present in long-term PCB-contaminated soil, evidenced by an increase in bacterial biomass after 6 weeks of incubation (Stella et al., 2017). In this work, an increase in the relative abundance of the phylum *Firmicutes* and *Proteobacteria* was observed after the treatments with *P. ostreatus* and *I. lacteus,* respectively. It is important to note that these phyla encompass well-known genera with reported PCB-degrading ability, such as *Bacillus*, *Pseudomonas*, *Achromobacter,* and *Burkholderia* (Zentetno-Rojas et al., 2020, Stella et al., 2017)*.* Even though the bacterial biomass increased, lower Chao1 and Shannon diversity indexes were determined after treatments. These indexes estimate the total quantity, diversity, and relative abundance of species in a sample, and a decrease in these indexes indicates an overrepresentation of a few phyla in the sample after treatment. It is well known that PCBs contamination reduces bacterial richness due to the inability of certain individuals to develop tolerance to the contaminant (Zentetno-Rojas et al., 2020). In this regard, the effects of fungal bioaugmentation upon already adapted bacterial communities should be evaluated in future investigations.

This shows that the mycoremediation of these compounds is complex, and factors affecting its effectiveness should be evaluated to optimize the process according to the presented conditions (i.e. matrix, concentration, chemical composition, and fungal strain).

Field application of mycoremediation techniques

Numerous studies in the field of bioremediation have demonstrated the effectiveness of fungal strains in eliminating PCBs and various other xenobiotics. Table 1 provides an overview of research conducted on WRF for bioremediation of organic pollutants. However, translating laboratory findings to real-world applications is challenging, and discrepancies between artificially contaminated matrices and real contaminated sites can be a problem when considering these approaches.

Several studies have investigated the use of these fungal strains for bioremediation of soil contaminated with PCBs. Stella et al. (2017) evaluated the potential of two WRF strains (*P. ostreatus* 3004 CCBAS 278 and *I. lacteus* 617/93) in degrading PCBs in long-term contaminated soil, as well as a biostimulation approach using lignocellulosic substrate for enhancing biodegradation. The fungal strains were immobilized using the same lignocellulosic substrate for soil treatment. Figure 3 shows a model workflow for the microcosm preparation for *in situ* mycoremediation and biostimulation experiments. After twelve weeks of incubation, *P. ostreatus* showed higher removal rates of PCBs compared to *I. lacteus* in top-soil and rhizosphere soil (50.5% and 41.3% respectively), while both species exhibited lower removal rates in bulk soil (18.5% for *P. ostreatus and* 19.3% for *I. lacteus*). Compared to bioaugmentation experiments, biostimulation resulted in a lower overall removal rate, achieving only a 22.9% removal rate for topsoil. Despite the removal rates obtained, the toxicity varied widely after treatment. Little reduction was observed on bulk soils, only bioaugmentation treatments reduced toxicity on topsoils, and all treatments increased toxicity within rhizosphere soils after treatment.

The same species of WRF (*P. ostreatus*, strain KRYOS) was also applied for the bioremediation of contaminated groundwater and surface water obtained from an excavation site with PCB contamination (Šrédlová et al., 2020). SMS, which contains viable mycelium and serves as inoculum for bioremediation strategies, was utilized in a trickle bed pilot-scale bioreactor for the biodegradation experiment. Figure 4 shows a scheme of the pilot-scale bioreactor used for this experiment. The pilot-scale bioreactor had a flow rate of 20 L/h and achieved an average removal of 89±9% of PCBs from the contaminated water, with concentrations ranging between 0 and 0.12 μg/L for different PCB congeners. No toxicity

effects were observed in the effluent from this treatment. A similar type of bioreactor using SMS derived from *P. ostreatus* production was applied to remediate sites contaminated with PAHs, resulting in a high depletion rate of 97.9% after 8 months (Di Gregorio et al., 2016). Another similar pilot trickle-bed reactor using *P. ostreatus* HK 35 showed promising results during its application for remediation at a wastewater treatment plant containing endocrine-disrupting substances; achieving a remarkable removal rate exceeding 97% within just 24 h (Křesinová et al., 2018). These examples show removal rates comparable to those attained by conventional remediation techniques (Šrédlová et al., 2022). Moreover, applying inexpensive fungal substrates, like SMS, significantly reduces operational costs associated with PCBs remediation (Šrédlová et al., 2020). These cost-effective approaches offer the potential for high removal rates without the negative environmental implications associated with other traditional methods, such as the generation of unwanted volatile compounds or the use of potentially toxic substances. Furthermore, these approaches could provide a solution for countries without access to specialized facilities for treating contaminated materials.

Nevertheless, implementing a mycoremediation approach on real contaminated sites represents a significant challenge due to the variability and complexity of composition across the contaminated matrix. One of the factors that can lead to these discrepancies is the bioavailability of PCBs in long-term contaminated soils. As contaminants remain in soil or sediment, they become less bioavailable possibly due to a decrease in bioaccessibility, i.e. a reduction in the freely dissolved and easily desorbed fraction of contaminants (Portet-Koltalo et al., 2020). Taylor et al., (2019) studied the effect of aging on the bioaccessibility of PCBs in marine sediment finding that not only the aging of contaminants resulted in a lower bioaccessibility, but the hydrophobicity of different PCBs was a determinant factor in their disponibility. They discovered that more hydrophobic congeners (PCB 153 - log K_{ow}: 6.9) were less bioaccessible than less hydrophobic congeners (PCB 52 - log K_{ow}: 5.8), being the average value for desorbable fractions of 0.4-1.5% for PCB 153 and 19.6–26.6% for PCB 52. They also found that contaminants were less bioaccessible in sediments with high organic matter. These findings agree with those reported by Portet-Koltalo et al., (2020), who concluded that the organic matter present in the sediment was the most influential factor that affected the extractability of PCBs and PAHs in contaminated sediments. To overcome this challenge, most strategies consider using biosurfactants to change contaminant solubility, which could increase contact area and bioavailability (Lászlová et al., 2018). In a bioaugmentation assay of a contaminated sewage canal with the bacterial strain *Achromobacter xylosoxidans*, the addition of biosurfactant saponin and rhamnolipids R-90 increased the PCB biodegradation by 55 and 60%, respectively (Lászlová et al., 2018). Some WRF strains, like *I. lacteus* BAFC 1171, can produce biosurfactants in the presence of PCBs (Sadañoski, Benitez et al., 2020) further supporting the role of this fungal group in remediation techniques. Employing strains capable of producing surfactants naturally contributes to lessening the environmental impact by avoiding the use of synthetic surfactants that may be toxic to other organisms (Badmus et al., 2021).

Also, different compositions in terms of concentration and congeners of PCBs in contaminated soil greatly influence removal percentages and toxicity reduction in mycoremediation. As shown by Stella et al., (2017), despite its high bioaccessibility (96%), the evaluated treatments (biostimulation and bioaugmentation) were not effective in the bioremediation of soils with high PCBs concentrations. The diversity in congeners also affects mycoremediation effectiveness, as higher chlorine content makes PCBs more resistant to fungal metabolism (Covino et al., 2016; Čvančarová et al., 2012). On the remediation treatment of contaminated water (Šrédlová et al., 2020) no degradation of hexa- or heptachlorinated congeners was observed. Additionally, it is important to note that certain subproducts of fungal metabolisms over PCBs can be more toxic than their parental compounds; for example, hydroxylated-PCBs exhibit higher toxicity derived from greater affinities for the aryl hydrocarbon receptor (Kamata et al., 2009). The production and accumulation of CBAs in metabolism should also be considered, as they can increase toxicity. These metabolites were found during PCBs biodegradation under laboratory conditions using WRF. For example, 4-CBA was detected from the degradation of 4,4′-dichloro biphenyl by two

strains of *Phanerocaete* (Kamei, Kogura et al., 2006). Similarly, hydroxy- and methoxy PCBs and their derivatives were determined during Arochlors 1242, 1254 and 1260 degradation by various WRF strains (Čvančarová et al., 2012), while trace levels of hydroxy and metoxy PCBs were found along with a significant proportion of 2,3,6- triCBA, a highly recalcitrant intermediate that resisted fungal degradation (Stella et al., 2013). This resistance to fungal degradation may be due to the double ortho-chlorine substitution and the electron-withdrawing effect of these chlorine atoms adjacent to the carboxyl group could prevent enzymatic attack (Stella et al., 2017). Considering these factors is crucial for understanding how remediation strategies impact the final reduction in toxicity during mycoremediation efforts.

As previously stated, several fungal species were evaluated for PCBs' mycoremediation in the last decades. Strains from the *Pleurotus* genus are reported as the most efficient PCB degraders, specifically *P. pulmonarius* LBM 105 (Argentina), *P. ostreatus* 3004 CCBAS 278 (Czech Republic), and *P. ostreatus* KRYOS (Sylvan©). These last two are closer to a real application, considering that they were already tested for the treatment of long-term contaminated soils and water (Stella et al., 2017; Šrédlová et al., 2020). Degradation by intra- and extracellular enzymes is their primary degradation mechanism, and their effectiveness is attributed to both enzyme versatility and high tolerance towards PCBs (Sadañoski et al., 2019; Benitez et al., 2021). These fungal species are globally distributed and adapted to diverse environmental conditions across different regions (Törős et al., 2022). Additionally, they have the advantage of being edible mushrooms, accounting for around 25% of the globally cultivated mushrooms (Raman et al., 2021), enabling the possibility of coupling their commercial production to mycoremediation processes, reducing their cost.

Finally, several factors must be considered when deciding whether to apply an *in situ* or *ex situ* mycoremediation strategy. As previously mentioned, *ex situ* strategies are more convenient when the quick removal of contaminated material is needed, and should account for the material transportation as a possible contamination source. Currently, *ex situ* mycoremediation is performed in pilot-scale bioreactors (Di Gregorio et al., 2016; Křesinová et al., 2018; Šrédlová et al., 2020), which can be easily accessed by most countries. As previously mentioned, these strategies can achieve considerably high PCBs removal rates (up to $89 \pm 9\%$ - Šrédlová et al., 2020). Additionally, some advances are being made in the design of new processes for the mycoremediation of PCBs. Sadañoski, Benitez et al., (2020) proposed a bioprocess flow sheet for the mycoremediation of contaminated mineral oil and calculated the distribution for energy consumption. This bioprocess requires 1573.81 MJ/ton of energy, comparatively lower than incineration processes (8988 MJ/ton) (X. Hu et al., 2011). Also, some works are developing the up-scaling of fungal and bacterial bioprocesses using computational frameworks that should allow for better modeling of the effects of adding, removing, or modifying molecular components and/or metabolic pathways (Wang G. et al., 2020). These approaches will serve for the development of more accurate and efficient PCBs mycoremediation bioprocesses. Although considerably cheaper than classical remediation strategies, *ex situ* mycoremediation methods still contain a higher operational cost than *in situ* approaches, mainly due to the energy cost associated with bioreactors.

In situ mycoremediation techniques often only require adding a substrate or nutrients to the contaminated material alongside the fungal strain to be effective. Applying *in situ* mycoremediation strategies carries with it the benefit of potentially stimulating the bacterial communities present in the contaminated site, further enhancing the strategy's effectiveness overall (Dou et al., 2024; Winquist et al., 2014). However, the removal rates are usually lower than bioreactor-based strategies, e.g., 50.5% PCBs removal with *P. otreatus* (Stella et al., 2017) or 29% using indigenous Ascomycete strains (Germain et al., 2021). Since most of the *in situ* approaches that assess PCBs removal by fungal strains are carried out in micro and mesocosms, there could be some variation when these approaches are effectively implemented on contaminated sites. Also, as previously discussed, the variation in terms of concentration, bioavailability, and variability of PCBs on the site could directly affect the

efficiency of *in situ* strategies, resulting in lower removal rates. Consequently, more studies are needed regarding *in situ* PCBs mycoremediation approaches that are carried out on contaminated sites to comprehend the limitations of the application of said strategies.

Biodegradation Pathways and Metabolites

When analyzing the potential pathways for PCB biodegradation carried out by WRF, we can highlight two distinct groups of enzymes: the extracellular ligninolytic fungal enzymatic system and the internal xenobiotic metabolism. The lignin-degrading system is nonspecific, non-stereoselective, and free-radical based, which allows WRF to degrade a wide variability as well as compound mixtures of contaminants (Chandra et al., 2021).

The LiP achieve the oxidation of the substrate by electron transfer coupled with H_2O_2 consumption, leading to a cleavage of the β-O-4 linkage in the phenolic structures (Cajnko et al., 2021). These enzymes' high redox potential and two single-electron transfer steps allow them to oxidase compounds such as phenolic compounds, amines, aromatic ethers, and polycyclic aromatics (Cui et al., 2022).

Fungal oxidase enzymes, especially Lac, have been extensively studied for their ability to act against aromatic compounds. These phenoloxidases can oxidatively dechlorinate PCBs-related compounds, hydroxyPCBs, and coplanar PCBs (Chelaliche et al. 2021), thereby reducing the toxicity of PCBs by dechlorinating the chlorine groups in non-ortho position and decreasing aryl hydrocarbon receptor-mediated toxicity (Loganathan & Masunaga, 2020). Lac catalyze the oxidation of a wide range of aromatic substrates while reducing molecular oxygen to water, being able to oxidize non-phenolic substrates via mediators such as 2,20-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) (Cajnko et al., 2021).

One of the earliest studies utilizing this enzyme for PCB transformation focused on the dechlorination and detoxification of monochlorobiphenyls by Lac produced by *Pycnoporus cinnabarinus* (Schultz et al., 2001). An enriched Lac (500 nmol mL^{-1} min^{-1}) was capable of converting 97% of the 3-chloro-4-hydroxybiphenyl, 89% of the 4-hydroxy-49-chlorobiphenyl, and 92% of the 2-hydroxy-5-chlorobiphenyl after 24 h. The primary product formed during this transformation was a dimer (5,5'-di-(2-hydroxybiphenyl)) with the consequent elimination of the chlorine atoms.

The ligninolytic fungal enzymes were further studied *in vitro* by Šrédlová et al. (2021). The enzymatic extracellular extract of the WRF strains *P. ostreatus* 3004 CCBAS 278 and *I. lacteus* 617/93 was obtained, and their study focused on the action of Lac, MnP, Manganese-independent peroxidase (MiP), and LiP. The effects of these enzymes on hydroxylated PCBs were examined, with results showing that Lac and MnP could degrade a wide range of hydroxylated PCBs, especially when mediated by substances such as syringaldehyde in the case of Lac. This was also confirmed by the presence of monochlorinated CBAs, which could be produced by the rupture of the biphenyl molecule. Additionally, it was observed during this study that Lac were also able to form dimers and quinones while mediating the dechlorination of PCBs, similar to findings observed in other works (Fujihiro et al., 2009; Kordon et al., 2010; Schultz et al., 2001). The MnP and MiP were able to degrade all of the chlorobenzaldehydes in the presence of glutathione (Šrédlová et al., 2021). Furthermore, MnP and MiP demonstrated the capability for degrading all chlorobenzaldehydes in the presence of glutathione. The degradation process varied significantly for chlorobenzyl alcohol removal; compounds with chlorine atoms in ortho positions proved resistant to enzymatic degradation, indicating that chlorine substituents located in ortho-position relative to functional groups impede the reaction. Interestingly, no LiP activity was detected during this work.

A proteomic study of PCBs degradation by the strain *P. pulmonarius* LBM 105 revealed differential expression of Lac, versatile peroxidases, and several other fungal oxidoreductases

(Chelaliche et al., 2021). This strain has exhibited a variety of Lac that are expressed in the presence of PCBs (Sadañoski et al., 2019), possibly induced by xenobiotic response elements present in promoter regions of different WRF Lac (Soden & Dobson, 2001).

Apart from the ligninolytic enzymatic system, WRFs can modify contaminants through a cellular mechanism known as xenobiotic metabolism, which plays a key role in maintaining cellular homeostasis during exposure to foreign compounds. This metabolism is carried out by enzymes with broad specificity, some of which are induced by the presence of specific contaminants. The structure and amino acid sequence of these biotransformation enzymes can vary between individuals, leading to differences in the rates at which these compounds are metabolized (Parkinson & Oglilvie, 2008). In phase I of this metabolism (Esteves et al., 2021), cytochrome P450 catalyzes the first steps in enzymatic biotransformation, resulting in monohydroxylated metabolites (J. Liu et al., 2020; Stella et al., 2013). This enzymatic family is capable of carrying out hydroxylation, epoxidation, dealkylation, sulfoxidation, deamination, desulfurization, dehalogenation, and nitro reduction reactions (Lin et al., 2022), thereby transforming PAHs (Young et al., 2015), insecticides (Mori et al., 2021), and benzoates (Matsuzaki & Wariishi, 2005). Glutathione S-transferase is another enzyme involved in the later stages of xenobiotic metabolism and in the transformation of PCBs. This enzyme is induced in the presence of PCBs during the degradation of these contaminants by WRF (Chelaliche et al., 2021). These proteins conjugate the tripeptide group glutathione to xenobiotics that are hydrophobic and present electrophilic atoms (such as chloride). In fact, if the xenobiotic contains sufficient electron-withdrawing atoms, direct conjugation can occur without prior modification of the compound (Parkinson & Oglilvie, 2008), as observed by Song et al. (2009) where glutathione reacts with chlorinated PCB-quinones via nonenzymatic, nonreductive nucleophilic substitution reaction, resulting in a glutathionylated quinone with associated loss of chlorine.

Regarding the study of the metabolites produced from fungal metabolism, some of the early work that faced the identification and characterization of intermediate products produced by fungal degradation of PCBs found two degradation products, 4-CBA acid and 4-chlorobenzyl alcohol, when analyzing the degradation potential of *P. chrysosporium* towards 4,4'-Dichlorobiphenyl, 3,3',4,4'-Tetrachlorobiphenyl, and 2,2',4,4',5,5'-Hexachlorobipheny (Dietrich et al., 1995). Kamei, Kogura et al. (2006) used two *Phanerochaete* strains to degrade 4,4′-dichlorobiphenyl and evaluate the degradation products. In this work, they found several hydroxy- and methoxy PCBs, as well as 4-CBA, 4-Chlorobenzyl alcohol, 4-Chlorobenzaldehyde, and 4-CBA methyl ester. These findings contributed to proposing a degradation pathway for PCBs by the strain of *Phanerochaete* sp. MZ142, in which the fungal P450 monooxygenase was initially involved in producing hydroxylated intermediates. They also suggest that the ligninolytic extracellular enzymes were possibly involved in the ring fission of the PCBs.

In 2012, Čvančarová et al. expanded the knowledge regarding the WRF metabolism on PCBs. Using evidence from the works of Kamei, Kogura et al. (2006), Kamei, Sonoki et al. (2006), and Muzikář et al. (2011), they proposed a general degradation pathway for WRF. The initial hydroxylation by fungal cytochrome P450 is also proposed, whereas the consequent cleavage of the aromatic structure is proposed to be due to ligninolytic enzymes. These works deepen the characterization of the production of CBAs during the metabolic degradation of PCBs. At least 12 types of CBAs were found (Muzikář et al., 2011) along with methoxy and hydroxy derivatives together with the reduced forms of the original acids, suggesting a possible action of alcohol-dehydrogenase, aryl-aldehyde dehydrogenase, and also cytochrome P-450 towards the transformation of the carboxylic group of CBAs (Čvančarová et al., 2012).

Finally, with the increasing evidence regarding the enzymatic activity towards PCBs and their metabolites, a final degradation pathway was proposed by Šrédlová et al. (2021).

From this pathway, the first step in the fungal metabolism is carried out by fungal Lac, which can break or dimerize the biphenyl molecule. Schultz et al. (2001) were the first to describe the dimerization of PCBs by Lac, with or without dechlorination, and mainly depended on the position of the chlorine atoms relative to the hydroxyl group on OH-PCBs. The cleavage of the biphenyl molecule by Lac is also conditioned by the presence of mediators and the quantity and position of chlorine atoms. Congeners with fewer chlorine substituents were more rapidly removed, and the presence of chlorine atoms surrounding the hydroxyl group reduced the action of laccase over OH-PCBs (Šrédlová et al., 2021). Following this initial rupture, the cytochrome P450 would be the first to oxidize the intermediate product of the biphenyl rupture. Stella et al. (2013) analyzed the degradation of CBAs *in vivo* and *in vitro* by purified Lac, MnP, and microsomal fractions of *Lentinus tigris,* finding that neither MnP nor Lac were able to oxidize CBAs, even in the presence of mediators. However, the microsomal fraction containing cytochrome P-450 monooxygenases transformed mono-, di-, and tri-CBAs. Following this, other fungal oxidases like MnP or MiP would continue to modify the different intermediates that are being produced by fungal metabolism. Some metabolites, like chlorobenzaldehydes, can't be efficiently degraded by Lac but can be highly removed by MnP and MIP in the presence of glutathione, achieving nearly a 90% of removal for 2,3-CB-CHO, 2,4-CB-CHO, 2,5-CB-CHO, 3,4-CB-CHO, and 3,5-CB-CHO (Šrédlová et al. 2021). Figure 5 shows a summarized metabolic pathway for biotransformation of PCBs using the previously mentioned evidence.

It is important to note that some of the metabolites produced during bioremediation strategies are less persistent in the environment than its PCB parent compound and can be further metabolized by other organisms present in the environment. This is the case of CBAs that are susceptible to degradation by bacteria such as *Burkholderia xenovorans* or plants such as *Armoracia rusticana* and *Solanum nigrum* (Ding et al., 2024). However, since CBAs are more soluble in water than their parent compound, they can enter water bodies from contaminated soils, and their toxicity towards the aqueous organism has already been proven (Samadi et al., 2020). The environmental fate of metabolites is a necessary consideration when searching for applying a mycoremediation strategy, and there is a lack of information regarding long-term studies after applying these strategies since most works focus only on achieving an efficient removal of the contaminants.

Challenges and Future Perspectives

Although PCBs production was banned many decades ago, information regarding their use, location, and environmental fate is still missing. Since its adoption in 2001, the Stockholm Convention has 186 parties aiming to phase out in-use PCBs by 2025 and ensure environmentally sound waste management by 2028 (https://www.pops.int/Home/tabid/2121/Default.aspx). However, only 13% of the signing parties (23 countries) have a full inventory and moved towards environmentally sound management. These countries include the larger producers of PCBs and are, with a few exceptions, considered "high-income" with the economic capacity to enact the proposed guidelines. In this regard, the USA represents a particular case, being the largest PCBs producer and one of the few countries that has not ratified the Stockholm Convention. Although they banned their production and use, they do not have national legislation for its management and elimination, resulting in incomplete records (Melymuk et al., 2022). Hence, the first challenge is the need for stronger policies and resources to create full inventories by assessing legacy PCBs, contaminated equipment, unintentional production, and possible leakages.

Some investigations have addressed the distribution of PCBs in the environment. Zhu et al. (2022) studied PCBs' spatial and temporal distribution in China. As expected, PCBs concentration was higher near electronic waste dissembling areas and industrial clusters. Significant concentrations were found in different environments, plants, animals, and tissues,

particularly in populations that lived near these e-waste areas. It was determined that the highest PCBs concentration of high chlorinated congeners occurred in soils and sediments. Given the chemical characteristics of PCBs, they are easily sorbed to soil and sediments and transferred from air to soil through atmospheric deposition (Gabryszewska & Gworek, 2020; Zhu et al., 2022). Thus, an approach to the PCBs issue should be to develop strategies prioritizing soil and sediment treatment.

Soils containing mixed contamination pose a challenge for PCBs treatment. Co-contamination of multiple organic compounds and metal(oid)s is often found near industrial sites. The co-occurrence of pollutants can complicate treatment efficiency due to interactions leading to changes in solubility, bioavailability, competition for binding sites, and inhibition of microbial metabolism (Gil-Díaz et al., 2022). A few authors have approached this issue by studying PCBs degradation levels by plants and bacteria in soils/sediments co-contaminated with Cr, Pb, phenanthrene, polybrominated diphenyl ether, and tetrabromobisphenol A (Wang et al., 2015; Huang et al., 2022; Gil-Díaz et al., 2022; Xu et al., 2024). However, these studies are still scarce, and reports of effective mycoremediation strategies that promote the simultaneous degradation of pollutants are currently unavailable.

Bioaugmentation with fungi can be a suitable method for the bioremediation of contaminated soils. One of the main limitations of this strategy is the ability of the fungal strains to tolerate and survive in the environment, especially WRF (Han et al., 2022). However, it has been proven that this issue can be overcome by amendments or fungal immobilization in low-cost materials, which could also increase tolerance towards environmental variations (Antón-Herrero et al., 2023; Kaewlaoyoong et al., 2021; Sadañoski, Tatarin, et al., 2020).

Another commonly cited disadvantage of bioremediation approaches is the time of treatment, which is known to be slower than chemical/physical treatments (Han et al., 2022). However, bioremediation techniques are usually more simple and less expensive. In this sense, although it requires more time, it can be a suitable approach for countries that do not have the economic/technological resources for PCBs treatment (Melymuk et al., 2022) and would represent a step toward the environmental management of these pollutants.

Moreover, mycoremediation can be combined with other bioremediation strategies. Lu et al. (2014) applied a combination of phytoremediation, mycoremediation, and vermiremediation to a former contaminated transformer and capacitor storage site. They found that the synergistic action of ryegrass (*Lolium perenne* L.) and the arbuscular mycorrhizal fungi *Glomus caledoniun* L. increased the PCBs removal rate by 15.9%, compared to the action of ryegrass alone, and the application of ryegrass, *Glomus caledoniun* L. and earthworm (*Eisenia foetida*) increased the removal rate by 21.1%. In this case, not only the secretion of root exudates may accelerate the growth of the degrading microorganisms and help to improve PCB availability for biodegradation, but the presence of earthworms may contribute to the fungal colonization rate of ryegrass roots, while mycorrhizal fungi could enhance the availability of N, P, and K contents in the soil and their uptake by the plant, resulting in improved plant growth. There have also been advances in the co-cultivation of algae with filamentous fungi, with potential application in wastewater treatment (Chu et al., 2021). Although these approaches possess great potential for the remediation of contaminated sites, they are scarcely studied (Ahmad et al., 2024). Focusing future research on the combined application of bioremediation strategies should help surpass some limitations common to these methods when applied independently.

Alternatively, the use of genetic engineering to achieve the heterologous expression of fungal enzymes with PCBs degradation potential can be used to overcome the limitations of some strains in terms of enzyme production. In this context, selecting the heterologous hosts would directly affect the enzyme activity and property because post-translational modifications are host-specific (Gaber et al., 2020). A Lac from *T. sanguineus* was

successfully expressed in *Trichoderma atroviride* (Balcázar-López et al., 2016). This heterologous Lac showed similar biochemical properties to the wild-type enzyme and could better degrade Bisphenol A, benzo[α]pyrene, phenanthrene, and four different dyes. Similarly, Zhang et al. (2023) enhanced the production of a *Trametes* sp. Lac in *Trichoderma reesei* by replacement of the *cbh1* locus by a *lacA* gene. They obtained a significant rise in Lac activity (168.3 U/L) when replacing this endogenous protein-encoding gene, probably due to a reduction in the endoplasmic reticulum stress, shown by a decrease in the expression of genes involved in the endoplasmic reticulum-associated degradation (ERAD) (Q. Wang et al., 2020). Analogously, Lac from *P. ostreatus* were heterologously expressed in *Aspergillus niger*, obtaining a notable increase in the production of this enzyme (60,000 U/L) compared to the native production of *P. ostreatus* (500 U/L). This heterologous also maintained the ability to oxidize bisphenol A. The heterologous expression of enzymes involved in PCBs metabolism, such as Lac, contributes to new fields of study regarding the applicability of alternative hosts in bioremediation. However, more studies regarding the action of these heterologous enzymes are needed to ensure that they can maintain the affinity and degradation potential against PCBs.

Another aspect to consider is the (non-)existing regulations regarding mycoremediation strategies. The United Nations Environment Programme (UNEP), the United Nations Industrial Development Organisation (UNIDO), and the Food and Agriculture Organization of the United Nations (FAO) have established thorough regulations and guidelines about PCBs management, disposal, and possible treatments (FAO, 2018; BCRC-Caribbean, 2020). These guidelines recognize bioremediation as an available method for PCBs cleanup, particularly for soil restoration. So far, there are no specific regulations or guidelines for the remediation of contaminated sites. The U.S. Environmental Protection Agency (EPA) has proposed *Principles for Greener Cleanups,* which outlines policies for management practices (Simon, 2020). They mention that spent-mushroom compost can be considered suitable for bioremediation, supporting the need for further research. It also states that the design of successful bioremediation systems relies on proper bench-scale treatability tests with samples from the target area, followed by onsite pilot tests. Optimization of initial bench-scale designs allows the determination of possible metabolic products, degradation mechanisms, effect on native microbiota, potential delivery methods, and any need for amendments or supplemental technologies.

In this context, the major challenge of mycoremediation of PCBs contaminated matrixes is the lack of information on field or upscaling applications (Han et al., 2022; Sharma et al., 2018). According to the reviewed studies, the biotreatment of liquid wastes can be conducted under submerged culture (SC) in bioreactors. Bioreactors are commonly used to produce fungal bioactive compounds; hence, various designs are already available. However, they still need improvement to handle larger working volumes (Ferreira et al., 2020). The fact that the most promising strains are filamentous fungi represents another issue, considering that their morphology under SC is variable (Patel et al., 2023). In this regard, it is possible to control it by setting the culture parameters, and some authors suggest that using pellets can facilitate handling (Ferreira et al., 2020). For solid matrixes, the use of solid-state cultivation (SSC) is considered a plausible approach for *ex situ* treatment. In this case, fungal morphology is no longer an issue, and the use of low-cost lignocellulosic materials as culture media is already established, reducing cultivation costs (Gomes et al., 2023). The main drawback is the lack of suitable bioreactor designs for SSC. In recent years, computational design and AI have emerged as tools that could be helpful in improving bioreactor designs for both SC and SSC (Bhardwaj et al., 2022). *In situ* bioaugmentation is also considered suitable for field application, particularly for soils. As previously discussed, several factors must be accounted for to ensure fungal growth in soil (e.g., soil characteristics and fungal strain) (Merino-Restrepo et al., 2020). We believe that the insufficient information regarding field application may be due to two main factors: research cost and time. Nonetheless, considering mycoremediation's advantages, we expect that field application studies will be available in the following years.

The fungal PCBs metabolism of WRF is a complex mechanism where both intracellular and extracellular enzymes are needed. Over the years, several studies have tried to elucidate the components of this metabolism, relating the metabolomic data to the enzymatic expression to create a general metabolic pathway that explains the PCBs transformation by these strains. However, there is still much to be discovered regarding the role of responsible enzymes, the differential affinity of enzymatic isomers, genetic expression, and long-term consequences. A better understanding of the regulation of the genes of key enzymes will allow us to design more efficient strategies and enable the use of heterologous expression to overcome the drawbacks related to working with strains that depend on lignocellulosic substrates to grow. Finally, clarifying the long-term impact of these techniques will help determine the real applicability of mycoremediation.

Conclusion

Mycoremediation has proven to be a technology with great potential for its application in matrices contaminated by various pollutants, including highly recalcitrant and toxic contaminants such as PCBs. WRFs are a highly utilized group for this type of strategy since the results in terms of degradation and detoxification have been encouraging. Now, these technologies face the challenge of the specific characteristics of each contaminated site, accompanied by a lack of information on field application. Therefore, along with a deeper study on fungal strains with bioremediation capacity, it is necessary to carry out extensive monitoring tasks in soils and sediments contaminated by PCBs to design the best approach for their remediation and recovery. Finally, the existing studies show that mycoremediation strategies can be effective but must be tailored for each contaminated site, considering existing regulations, economic constraints, and possible environmental impact. In this context, governments must update regulations regarding this matter and support further research to achieve solutions that are suitable for their particular region.

Acknowledgments

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Legends

Figure 1. Classification of the primary technologies utilized for PCBs remediation in soil and sediment.

Figure 2. Advantages of using WRF for mycoremediation strategies. The central image represents the *Pleurotus pulmonarius* species and was obtained from wikimedia commons (Pleurotus pulmonarius 134572257.jpg).

Figure 3. Workflow of the microcosm preparation for the bioremediation and biostimulation experiments carried out by Stella et al., (2017). Sterilized wheat straw-based pellets were used as lignocellulosic substrates (LS). The moisture of the LS was adjusted to 75 %. The moisture of the soil samples was adjusted to 60 %. For each treatment the colonized LS was mixed with the soil to reach a final soil:LS ratio of 5:1.*) The two fungal strains used for these experiments were *Pleurotus ostreatus* 3004 CCBAS 278 and *Irpex lacteus* 617/93. Biostimulation experiments consisted in the same workflow but without the fungal inoculation of the LS.

Figure 4. Scheme of the pilot scale bioreactor used for the remediation of contaminated groundwater and surface water using spent oyster mushroom (Šrédlová et al., 2020). The fungal substrate consisted in a rehydratated (75 % humidity) wheat straw pellets with P. ostreatus KRYOS spent mushroom substrate (1:1, V:V).

Figure 5. Summarized metabolic pathway for PCBs biotransformation by WRF. This model is proposed based on the works of Chelaliche et al., (2021), Šrédlová et al., (2021), and Čvančarová et al., (2012). When possible, enzyme involvement is stated. Dashed lines correspond to steps that are yet to be clarified. All protein structure were obtained from *Protein Data Bank* (https://www.rcsb.org/).

CRedit Author Statement

Chelaliche Anibal Sebastian: Writing - Original Draft, Conceptualization, Investigation, Data curation, Visualization

Benitez Silvana Florencia: Writing - Original Draft, Data curation, Investigation, Visualization

Alvaregna Adriana Elizabet: Writing - Review & Editing, Supervision

Zapata Pedro Dario: Writing - Review & Editing, Supervision

Fonseca Maria Isabel: Writing - Review & Editing, Supervision, Project administration

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1. Examples of different WRF utilized for the remediation of diverse organic contaminants.

Fungal Strain	Pollutant	Initial Concentration	Removal percentage (%)	Reference
Trametes versicolor ATCC 42530	Naproxen (NPX) y Carbamazepine (CBZ)	0.096 mg/g	100 % for NPX and 48% for CBZ at 72 hs	Rodríguez-Rodríguez et al., 2010
Trametes versicolor ATCC 42530	Diclofenac (DCF), Naproxen (NPX), Indometacin (IDM), Ibuprofen (IBP), Fenoprofen (FEP)	10 µg/l	100 % for DCF, NPX, IDM, IBP y FEP after 48 hs	Tran et al., 2010
Trametes versicolor 167/93	Chlorobenzoic Acid (CBAs)	10 µg/ml	100 % for 3-CBA, 4-CBA, 2,3-CBA, 2,4-CBA, 2,5-CBA, 3,4-CBA, 3,5-CBA, 2,3,5-CBA at 21 days	Muzikář et al., 2011
Trametes versicolor 20148	Polycyclic aromatic hydrocarbon: Phenanthrene (PHE) y Pyrene (PYR)	100 mM	80 % for PHE and 70 % for PYR after 11 days	Rosales et al., 2013

Trametes versicolor BAFC 4272	Malachite green	50 μM	50 % of remotion after 2 hs	Kuhar et al., 2015
Trametes versicolor CDBB-H1051	Reactive Black 5 (RB5)	200 ppm	84 % remotion after 24hs	Martínez-Sánchez et al., 2018
Trametes versicolor ATCC 42530	Chlorpyrifos (CLP), Dicofol (DCL) y Cypermethrin (CPM)	5 μg/l	94,7 % for CLP, 87,9 % for DCL and 93,1% for CPM after 2 days	K. Hu et al., 2020
Trametes versicolor ATCC 42530	Polybrominated diphenyl ethers (PBDE)	5 mg/L	87 % for Deca-PBDE after 7 days, 67 % for octa-PBDE and 85 % for penta-PBDE after 14.5 days	Vilaplana et al., 2015
Trametes versicolor NBRC4937	BisphenolA (BPA), Butylparaben (BTPRB), Metylparaben (MTPRB), Nonylphenol (NP) y Dimethyl phthalate (DMPTL)	100 μM	100 % for BPA and BTPRB after 2 days, 100 % for MTPRB after 4 days, 100 % for NP after 8 days and 60 % for DMPTL after 8 days	Pezzella et al., 2017
Trametes versicolor DSMZ 3086	Synthetic Humic acids (HA) and real effluents	1 mg/L	80 % remotion for both effluents after 19 days	Zahmatkesh et al., 2018
Trametes versicolor 167/93	Delor 103	0.2 mg/ml	60 % remotion after 42 days	Čvančarová et al., 2012
Trametes sanguinea LBM 203	Arochlor 1242, 1254 and 1260	217 mg/L	59,86 % remotion after 24 days	Benitez et al., 2021

Irpex Lacteus	Delor 103	0.2 mg/ml	70 % remotion after 42 days	Čvančarová et al., 2012
Pleurotus ostreatus	Fluorene	30 mg/L	89,16 % remotion after 30 days	Akdogan & Pazarlioglu, 2011
Pleurotus ostreatus CECT 20600	Phenanthrene (PHE) y Pyrene (PYR)	100 mM	91 % remotion for PHE and 95 % for PYR after 11 days	Rosales et al., 2013
Pleurotus ostreatus *BWPH*	Brilliant green, Evans Blue and its mixture (1:1 p/p)	0,06 g/L for Brilliant green 0.15 g/L for Evans Blue, 0,08 g/L for the mixture	95,4 % remotion for Brilliant green, 53 % for Evans Blue and 83,6 % for the mixture after 96 Hs	Przystaś et al., 2013
Pleurotus ostreatus PO-3	Benzo-a-Pyrene	10 µg/mL	64,3 % remotion after 15 days	Bhattacharya et al., 2017
Pleurotus ostreatus 3004 CCBAS 278	Chlorobenzoic Acid	10 µg/mL	100 % for 3-CBA, 4-CBA, 2,4-CBA, 3,4-CBA, 3,5-CBA after 21 days	Muzikář et al., 2011
Pleurotus ostreatus ATCC MYA-2306	Bisphenol A (BPA), Butylparaben (BTPRB), Metylparaben (MTPRB), Nonylphenol (NP) y Dimethyl phthalate (DMPTL)	100 µM	70 % remotion for NP, 60% for BPA, BTPRB, MTPRB, DMPTL after 8 days	Pezzella et al., 2017
Pleurotus ostreatus *3004 CCBAS 278*	Delor 103	0.2 mg/ml	99,6 % remotion after 42 days	Čvančarová et al., 2012

Pleurotus ostreatus HK 35	Wastewater treatment plant effluent	40-80 µg/L of endocrine disrupting substances	97 % removal after 24 hs	Křesinová et al., 2018
Pleurotus ostreatus *spp florida G241*	PCBs contaminated soil	3000 mg/Kg	Over 90 % for Mono- ,Di-,Tri- and Tetrachlorobiphenyls. 60 % for Pentachlorobiphenyls and 30 % for Hexachlorobiphenyl after 35 days	Zeddel et al. 1993
Pleurotus ostreatus Strains 3004, F6, P15 and P19	Delor 103	1.021 mg/ml	40 % remotion after 2 months	Kubátová et al., 2001
P. ostreatus, strain KRYOS	Contaminated with the PCB mixture Delotherm	0.1-1 µg/L of PCBs	89 ± 9%remotion after 71 days	Šrédlová et al., 2020
Pleurotus pulmonarius LBM 105	Arochlor 1242, 1254 and 1260	217 mg/L	97,7 % remotion after 35 days	Sadañoski et al., 2019
Phanerochaete chrysosporium ME 446	Delor 103	0.2 mg/ml	60 % remotion after 42 days	Čvančarová et al., 2012
Phanerochaete chrysosporium BKM-F-1767	Acid Violet 7 and Fuschsine Basic	40 mg/L	93,4 % for Acid Violet after 3 days and 67,9 % for Fuschsine basic after 5 days	Li et al., 2015
Phanerochaete chrysosporium CGMCC 5.0766	Phenanthrene and Pyrene	0,33 mg/L for Phenanthrene and 0,033 mg/L for Pyrene	99,55 % remotion for Phenanthrene and 99,47 % for Pyrene after 60 days	Ding et al., 2013

Phanerochaete chrysosporium	Crude Oil	600 ppm	58,1 % remotion after 12 days	Behnood et al., 2014
Phanerochaete chrysosporium BKMF-1767	2,4-Dichlorophenol	50 mg/L	100 % remotion after 24 hs	Chen et al., 2014
Phanerochaete chrysosporium BKM-F1767	BisphenolA	250 mg/kg	58,23 % remotion after 24 days	C. Hu et al., 2018
Phanerochaete chrysosporium ME 446	Chlorobenzoic Acid	10 µg/mL	100 % for 3-CBA, 4-CBA, 2,3-CBA, 2,4-CBA, 2,5-CBA, 3,4-CBA, 3,5-CBA, 2,3,5-CBA after 21 days	Muzikář et al., 2011
Phanerochaete chrysosporium DSM 13583	Bisphenol A (BPA), Butylparaben (BTPRB), Metylparaben (MTPRB), Nonylphenol (NP) y Dimethyl phthalate (DMPTL)	100 µM	100 % for BTPRB, 100 % for MTPRB, 80 % for NP, 60% and 45 % for BPA y DMPTL after 8 days	Pezzella et al., 2017
Phanerochaete chrysosporium ATCC 24725	Diuron Herbicide	7 µg/mL	94 % remotion after 10 days	Coelho-Moreira et al., 2013
Phanerochaete chrysosporium BKM-F-1767	2,4,2,4-Tetrabromodiphenyl Ether	2 µg/mL	99 % remotion after 3 days	Z. Liu et al., 2018
Pycnoporus sanguineus 2126	Malachite green, Bromophenol Blue	50 µM	90 % remotion for Bromophenol Blue y 30 % for Malachite green after 14 days	Shimizu et al., 2009