Application of immobilized mycelium-based pellets for the removal of organochlorine compounds: a review
J. C. V. Pereira, M. P. Serbent and E. Skoronski

ABSTRACT
Organochlorines have diverse structures and applications and are included in the list of persistent organic pollutants (POPs) due to their toxicity and environmental persistence. The reduced capacity of conventional wastewater treatment plants to remove these compounds encourages the development of cost-effective and efficient remediation approaches. Fungal biotechnology can contribute to the development of these technologies through their enzymatic machinery but faces several drawbacks related to the use of dispersed mycelium. In this sense, investigations concerning the degradation of organochlorines using immobilized fungi demonstrated an increase in contaminant removal efficiency compared with degradation by free cells. Despite this interest, the mechanisms of immobilized fungi have not been comprehensively reviewed. In this paper, recent advances of laboratory and field studies in organochlorine compounds removal by fungi are reviewed, focusing on the role of immobilization techniques. Firstly, the mechanisms of organochlorines bioconversion by fungi and the factors affecting enzyme activity are elucidated and discussed in detail. Then, the main targeted compounds, fungi, technics, and materials used for immobilization are discussed, as well as their advantages and limitations. Furthermore, critical points for future studies of fungi immobilization for organochlorine removal are proposed.

Key words | bioconversion, fungal immobilization, novel materials, organochlorine compounds

HIGHLIGHTS
• Recent researches on fungal bioconversion of organochlorine compounds are introduced.
• Strategies for enhancing enzymatic activity through immobilization are outlined.
• Cost-effective and novel materials used for immobilization are listed.
• Simultaneous cultivation of fungi and bacteria to form immobilized biomixtures is graphically described.
INTRODUCTION

Because of the increasing contamination of the environment by persistent organic pollutants (POPs), it is imperative to develop cost-effective and efficient remediation approaches. Of these pollutants, organochlorine compounds include an expanding array of pesticides, medicaments, personal care products and estrogenic substances called ‘endocrine-disrupting compounds’ (EDCs) (Table 3). The wide distribution of these substances in the environment and their hydrophilic properties represent a threat to humans and aquatic life through drinking water (Kresinova et al. 2018). Indeed, the Global Monitoring Plan of the Stockholm Convention on POPs reported that information on gradual changes in concentrations of the 16 newly listed POPs (nine of them organochlorines) in water is still limited (UNEP 2017). In this sense, the scientific community has dedicated extensive research into mechanisms to remove organochlorine compounds from water resources.

These compounds can be degraded through biological and abiotic ways. However, bioremediation represents the most effective alternative to transform them into simpler and less toxic compounds, through the enzymatic machinery of fungi (Kumar & Pannu 2018). According to Barber et al. (2020), one of the most promising strategies relies on a combination of three families of extracellular lignin modifying enzymes (LME) consisting of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, which gives them the potential for oxidation of a wide variety of chemicals and even mixtures of chemicals. The intracellular enzymes, such as the cytochrome P450 system, also play a pivotal role in various fungal metabolisms, due to its unique and versatile biocatalytic properties (Hussain et al. 2020). Nevertheless, no distinct degradation effects on organochlorines have been achieved solely using pure enzymes (Marco-Urrea et al. 2009; Haugland et al. 2019). Hence, ongoing research currently focuses on mycelial-based degradation (whole-cell degradation) of pure or mixed cultures, considering that fungi are our present, and they will open new avenues for a future bio-based circular economy (Meyer et al. 2020).

The fundamental advantage of bioreactors based on fungal mycelium is their scale-up potential and their applicability in continuous reactors. However, dispersed fungi growth in submerged cultures usually exhibits limitations such as excessive growth, foaming and extra need for mixing and oxygen supply (Mir-Tutusaus et al. 2018a, 2018b). To overcome or reduce such limitations, recent studies have focused on fungi mycelium immobilization strategies.

The immobilization of fungi can be defined as the restriction of the free migration of cells, maintaining their metabolic, catabolic, and catalytic activities (Garcia-Reyes et al. 2017). The strategies involve various materials and support. Moreover, some filamentous fungi can grow as mycelial pellets (auto immobilization) in submerged cultures, which results in an adequate adsorption capacity and immobilization of microorganisms (Wang et al. 2019). Most fungi, especially white-rot fungi (WRF), cannot use organo-pollutants as sources of carbon and energy.
However, they require lignocellulosic substrates such as sawdust, flaxseed, straw and other such materials serving both as support and carbon source (Bekhit et al. 2018). This allows the use of a broad spectrum of materials for fungi immobilization. The technics of immobilization have been used mostly in dye removal (Sato et al. 2002; Yesilada et al. 2010; Wang et al. 2015). Still, studies addressing this issue are in progress.

The investigations into the degradation of organochlorines using immobilized fungi have reported increased removal efficiency compared to degradation via free cells. Nevertheless, most of the studies have been carried out in batch mode and at small scale. In this context, investigations into the effect of real wastewater conditions are necessary in order to propose safe and cost-effective applications to remove organochlorines in continuous mode (Badia-Fabregat et al. 2017; Mir-Tutusaus et al. 2019). While the use of immobilized extracellular enzymes for the degradation of organochlorines and other xenobiotics has been previously reviewed (Barber et al. 2020), information regarding the use of whole-cell immobilization for the same purpose is still scarce.

This work aimed to compile the most important aspects of biodegradation of organochlorine compounds by fungi, emphasising immobilization strategies. Moreover, the primarily targeted contaminants, the species of fungi and materials used, and issues related to the advantages and limitations of the immobilization techinics in different operational modes were reviewed.

Fungal bioconversion of organochlorine compounds

The unique ability of fungi to produce large amounts of specific and non-specific enzymes makes them very attractive for the degradation of organic pollutants (Jin et al. 2016; Kaur et al. 2016; Coelho-Moreira et al. 2018). Several strains of micro and macrofungi have been used to remove three main groups of pollutants: (1) dyes – widely used in the textile, printing, and paper industries; (2) pharmaceutical compounds; and (3) organochlorines. This last class of pollutants is mainly detected in wastewater that contains metabolized and non-metabolized medicaments. Besides, effluents from the pesticides, solvents, and pulp and paper industries are also important sources. The presence of these compounds in water is of great concern due to the low capacity of conventional wastewater treatment plants to remove them (Schenck et al. 2012; Wang et al. 2015; Wang et al. 2016; Chun et al. 2019; Dalecka et al. 2020). Although fungi-mediated removal of dyes and metals from wastewater has already been investigated (Forgacs et al. 2004; Yesilada et al. 2010; Mani & Kumar 2014; Cecchi et al. 2019), the application of fungi to remove organochlorine species requires further investigation. Specifically, mycelium-based treatment of pollutants involves the processes of biosorption and biodegradation. In biosorption, the pollutant binds to the fungal mycelium walls and can later be accumulated inside the cells, coming into contact with intracellular enzymes (Kulshreshtha 2019). Meanwhile, biosorption represents a crucial step in biodegradation, and many studies have achieved this process by immobilizing the fungal mycelium (Kulshreshtha 2019). According to studies involving immobilized fungi to remove organochlorines, biosorption depends on the culture conditions. This process is facilitated in a lower pH range, but it is commonly inhibited at pH below 3 (Behloul et al. 2017). A large concentration of H⁺ protons fully occupres the active sites of the biomass, inhibiting the ability of organochlorine biosorption by the fungal biomass (Behloul et al. 2017). This phenomenon was reported by Bosso et al. (2015), who studied the influence of concentration and pH on the biosorption of pentachlorophenol (PCP), using the species Anthracophyllum discolor in the form of pellets. For initial concentrations at 5 and 10 mg/L, the amount of PCP adsorbed was greater than 80% in pH range between 5.0 and 5.5. To increase the biosorption capacity, treatments can be applied directly to living or dead biomass (Bosso et al. 2015), but this is only the first step towards effective biodegradation of organochlorines. In addition to ensuring that the fungus can adsorb the contaminant in more realistic conditions, close to neutrality, the treatment applied needs to ensure good conditions for satisfactory enzymatic activity.

Most importantly, the enzymatic machinery of fungi allows the oxidation of a wide variety of xenobiotics. This ability is mainly linked to its system of non-specific extracellular enzymes, represented by lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Liu et al. 2019). A detailed list of the main classes of enzymes involved in fungal catabolism of organic pollutants was introduced by Harms et al. (2011). A study conducted by Palli et al. (2017), using the fungus Pleurotus ostreatus to remove diclofenac present in hospital wastewater, reveals the relevance of the laccase enzyme. When a fluidized bed reactor was used, total medicament degradation was achieved in 24 hours. In comparison, the same result was obtained in only 4 hours when purified laccase was used.

Table 1 lists studies demonstrating the action of the extracellular enzyme for the removal of organochlorine...
compounds under different conditions. The reports are mainly associated with the species *Trametes versicolor* and species of the genus *Aspergillus* (Pulicharla et al. 2018; Pylypchuk et al. 2018; Sarker et al. 2020). *Trametes* is a genus of fungi known to be one of the leading producers of laccase, and for this reason, most of the studies assess the role of this enzyme in the bioremediation process (Table 1). The activity of these enzymes follows a reaction mechanism between radicals, driven by the differential of redox potential between the active site of the enzyme and the substrate (Barber et al. 2020). Laccase catalysis is directly linked to the chemical structure of the substrate, while it is more expressive in compounds that have both donor and electron trapping functional groups. Some organochlorine compounds such as 2,4-dichlorophenol, diclofenac, and triclosan have a particular chemical structure, in which both functional groups are present. Spina et al. (2020) investigated the potential of enzymatic treatment via laccases obtained from the fungus *Trametes pubescens*. The authors measured the biodegradation of dichlorophenol, diclofenac, and triclosan detected in municipal wastewater. They observed contaminant removal from 76.8 to 80.3% through laccase oxidation. It is important to note in this case that the presence of more than one contaminant can help laccase

| Enzyme       | Compound/metabolite(s) | Treatment conditions | Fungal source | Reference                        |
|--------------|------------------------|----------------------|---------------|----------------------------------|
| Laccase      | 2-chlorophenol C0 = 300 μmol/L | Samples from municipal water resource recovery facility. Laccase/mediator system. Removal of almost 80% in 10 minutes. | *Trametes versicolor* | Haugland et al. (2019) |
|              | Chlortetracycline (CTC) C0 = 2.0 mg/L | CTC added in municipal wastewater. Removal of 87% of CTC by free laccase after 48 hours. | *Trametes versicolor* | Pulicharla et al. (2018) |
|              | 2,4-dichlorophenol, alachlor, diclofenac and triclosan C0 = 0.16–0.84 μg/L | The sample was collected from a municipal wastewater treatment plant after primary sedimentation and at the end of the process. 100 U/L of laccases were used to treat the pollutants. The biodegradation yields ranged from 40 to 80%. | *Trametes pubescens* | Spina et al. (2020) |
| Diclofenac (DCF)/acetaminophen mixture C0 = 25 mg/L | Aqueous media. Laccase immobilized on Fe3O4/SiO2-diethylenetriamine pentaacetic acid (DTPA) hybrid nanocomposites. Removal of 85% of DCF after 18 hours. | *Trametes versicolor* | Pylypchuk et al. (2018) |
| Dichlorophen (DCP) C0 = 0.1–10 μmol/L | In vitro assay. Laccase immobilized on synthetic mesoporous foam. Reduction of cytotoxicity and genotoxicity of DCP, after 48 hours. | *Coriolopsis gallica* | Vidal-Limon et al. (2018) |
| 3,5-dichloroaniline (3,5-DCA) C0 = 0.2 mM | Aqueous media. Laccase/mediator system. Complete degradation of 3,5-DCA in 24 hours. No assessment of the toxicity of the by-products generated using mediators, under the treatment conditions. | *Trametes versicolor* | Sarker et al. (2020) |
| Mn peroxidase | Triclosan (TCS) C0 = 5.0 mg/L | Insoluble enzyme. Biotransformation of 75% of TCS, in a fixed bed reactor with a residence time of 150 minutes. | *Ganoderma lucidum* | Bilal et al. (2017) |
|              | Diclofenac (DCF), indomethacin (IND) C0 = 2.0 mg/L | Medicaments added in enzymatic extract. The removal percentage showed a strong correlation with the specific enzymatic activity of MnP. Removal of 73% of IND by Pleurotus sp. and approximately 90% removal of DCF by *Trametes maxima*, after 1 hour. | *Trametes máxima*, *Pleurotus sp.* | Ravelo et al. (2020) |
| Chloroperoxidase | Ketoconazole (KTC) C0 = 20 μM | Medicament added in the effluent from the secondary treatment of a water treatment facility. Enzyme immobilized on chitosan macropores. Removal of 99% of KTC after 10 minutes. | *Caldaromyces fumago* | García-Zamora et al. (2018) |

*C0 represents the initial concentration of the target compound.*
activity. These contaminants together act as mediators and serve as a substrate for the enzyme to generate intermediates with high redox potential. However, Bilal et al. (2017) also reported degradation of the medicament triclosan by manganese peroxidase extracted from G. lucidum strains at almost ten times higher initial concentration.

The laccase activity in real wastewater is affected by natural organic matter, which is more susceptible to laccase oxidation than the target contaminant (Le et al. 2016). Therefore, some studies using laccase for organochlorine removal are performed in the presence of redox mediators and/or using immobilized laccase (Table 1). The use of mediators to increase the oxidative power of enzymes is not recommended, as it generates by-products that are to be treated later, as well as increasing the system costs. The degradation of 2-chlorophenol by laccase produced from Trametes versicolor showed the fastest results in the presence of a mediator, but generated five different by-products of mediator-phenol couples and polymers (Haugland et al. 2019). Alternatively, some authors replace mediators with physical processes such as ultrasonication (Pulicharla et al. 2018) or enzyme immobilization. Pylypchuk et al. (2018) enhanced the laccase oxidation using nanocomposites for immobilization, and even in an acid environment at pH 3, the enzyme activity was not affected. Similarly, the detrimental cytotoxic and genotoxic effects of the pesticide dichlorophen were significantly reduced after oxidation with laccase immobilized on mesoporous synthetic silica foam (Vidal-Limon et al. 2018). The immobilization of chloroperoxidase from Caldariomyces fumago also showed promising results in removing the medicament ketoconazole, in addition to increasing the efficiency of the process on a broader pH range (<5) (García-Zamora et al. 2018).

Target contaminants are mainly associated with medicaments, and among these, diclofenac has been the most reported. The typical concentration of the solutions is 2.0–25 mg/L, and authors use laccase enzymes and manganese peroxidase from the genus Trametes as biocatalyst (Pylypchuk et al. 2018; Ravelo et al. 2020; Spina et al. 2020). It was identified that, unlike manganese peroxidase, the enzyme laccase only degraded diclofenac when tested on its own (Ravelo et al. 2020). In general, the laccase enzyme has been the most used due to its versatility to oxidize many compounds with a more complex structure. Furthermore, the immobilization of extracellular enzymes has shown faster removals, while several studies report the possibility of enzyme reuse.

The fungi intracellular system is also involved in the biodegradation of other organochlorine compounds. The use of genetics and proteomics is essential to better understand the ability of fungi in the degradation of organochlorines, including the identification of relevant genes and intra-enzymatic interactions. Cytochrome P450, part of a large family of intracellular enzymes (CYP), plays an essential role in the degradation metabolism of fungi, catalysing vital monooxygenation reactions in primary and secondary metabolism, resulting in the production of several secondary metabolites. The diversity of CYP450 monooxygenase enzymes found in different fungi makes them adaptable to different ecological niches (Hussain et al. 2020). Recently, the genome sequence of the filamentous fungus Trametes trogii revealed an estimated 158 CYP genes, the highest number known until that time among the fungal genomes (Liu et al. 2019), suggesting a variety of metabolic functions. Table 2

---

**Table 2** | Studies where evidence of participation of intracellular enzymes in the biodegradation of organochlorines was found

| Enzyme | Compound/metabolite(s) | Treatment conditions | Fungal source | Reference |
|--------|------------------------|----------------------|---------------|-----------|
| CYP450 enzymes/ Cytochrome P450 monooxygenase | 2,4-dichlorophenol (2,4-D) | Flask experiments. 80% of 2,4-D removal, after 8 hours. The removal rate of 2,4-D decreased to 16% in the experiments supplemented with 1.0 mM of CYP inhibitor at the final time. | *Thielavia* sp. | Mtibaa et al. (2020) |
| | *C₀ = 22 mg/L* | | | |
| | Pesticide Endrin | Flask experiments. When 1.0 mM inhibitors were added in *P. brevispora* and *P. acanthocystis* cultures, the degradation rate decreased by 25 and 40%, respectively. | *Phlebia brevispora* and *Phlebia acanthocystis* | Xiao & Kondo (2019) |
| | *C₀ = 10 µM* | | | |
| | Medicament Lamotrigine (LTG) | Flask experiments. When 0.4 mM inhibitor was added to the medium, the degradation rate decreased by almost 40%. | *Pleurotus ostreatus* | Chefetz et al. (2019) |
| | *C₀ = 100 mg/L* | | | |

* C₀ represents the initial concentration of the target compound.
comprises several studies regarding the action of intracellular enzymes for the removal of organochlorine compounds under different conditions.

Xiao & Kondo (2019) reported a decrease in the degradation of the organochlorine pesticide endrin in the presence of CYP450 inhibitors. In strains of Phlebia acanthocystis, the rate of degradation decreased by 40% compared to cultures without inhibitors, and some reports suggest that CYP450 monoxygenases are involved in the first steps of chloro-organic pollutant degradation. Mtibaa et al. (2020) reported that the bioconversion of 2,4-dichlorophenol by the ascomycetous Thielavia sp HJ22 was preceded by a hydroxylation resulting in two products and catalysed mainly by CYP450 monoxygenases. The authors also observed the reductive dehalogenation of one of these products, catalysed mainly by cytochrome P450 monoxygenases, followed by an oxidative reaction by the enzyme laccase. Similarly, the bioconversion of the medicament lamotrigine by the white-rot fungi Pleurotus ostreatus was partially inhibited with the addition of two different cytochrome P450 inhibitors. In this case, the removal was not completely blocked, suggesting another additional mechanism of transformation, such as extracellular enzymes (Chefetz et al. 2019). The degradation efficiencies listed in Tables 1 and 2 do not demonstrate significant differences between extracellular and intracellular enzymes but suggest a relation of dependence of intracellular enzymes in the first steps of chloro-organics bioconversion. However, most of the results come from indirect studies using CYP450 inhibitors, and therefore, the involvement of intracellular enzymes in the biodegradation of organochlorine pollutants requires further studies.

Typically, organochlorine compounds are present in minimal amounts in water resources. Nonetheless, even small amounts can be detrimental to the aquatic ecosystem and represent a threat to humans. The concentration of the organochlorines 2,4-D, alachlor, dichlofenac and triclosan is 0.16–0.84 μg/L in municipal wastewater in Italy (Spina et al. 2020), and a relatively higher chemical load of dichlofenac was observed in hospital pre-treated (coagulation) wastewater in Spain (Mir-Tutusaus et al. 2019). In this sense, the fungal biodegradation of organochlorine compounds usually happens co-metabolically, and the second substrate is needed (Mir-Tutusaus et al. 2018a, 2018b).

The number of species that show a capacity to degrade organochlorines is vast and include a diverse phylum. These different fungi species promote several changes in the metabolic pathways to metabolize many organochlorine pollutants actively (Table 3). Reactions such as reductive dechlorination, N-dealkylation, hydroxylation, deoxygenation, and aromatic ring fission are performed using various fungi types.

These reactions are catalysed by their intracellular/extracellular enzyme system. To ensure satisfactory enzymatic activity and avoid some of the difficulties associated with the use of dispersed mycelia cells in continuous processes, immobilization techniques have been increasingly used. The use of dispersed mycelium in bioreactors faces several operational difficulties, such as excessive biomass growth in the reactor walls, increased oxygen supply, need of agitation, and foam formation (Krull et al. 2013; Antecka et al. 2016).

Technics for fungi immobilization

Biomass immobilization is performed through different techniques and varies according to the objectives proposed. Methods previously used in industrial and medicinal processes to obtain enzymes, antibiotics, organic acids, and other products were adapted for bioremediation (Gotovtsev et al. 2015). Self-immobilization in pellets and mycelium adhesion to a fixed or porous surface are the main methods employed. The first method is based on the natural tendency that some filamentous fungi species in submerged cultures develop a morphology in pellets (Gibbs et al. 2000). In other fungal species, the fixation to a surface or supporting material has a predilection due to exopolysaccharides (EPS) acting as an adhesive material. Immobilization in a supporting material is also performed by encapsulating the fungal mycelium in the pores or intersections of fibrous materials or physically trapped within porous solids or matrices (Garcia-Reyes et al. 2017). To date, only a few researches have focused on alternatives for the optimization of mycelial based treatment in the degradation of organochlorines using immobilization techniques. Two main types of processes have been used: biofilm formation using a support material and those based on physical retention via entrapment on porous matrices or membrane encapsulation (Sun et al. 2010; Garcia-Reyes et al. 2017). More recently, the use of fungal pellets has gained attention due to its adequate self-immobilization capacity and the possibility to combine another microorganism or material to form self-immobilized biomixtures (Yu et al. 2020). However, the specific mechanisms of immobilization for organochlorine removal are not fully understood.

Self-immobilization in the form of fungal pellets

Pellets are spherical or ellipsoidal mycelial granules or oval masses constituted by interlaced hyphae, generally in a size
Table 3 | Mechanisms of organochlorine compounds degradation by fungi

| Compound                        | Chemical structure | Mechanism of degradation | Fungi                                                                 | Reference                                                                 |
|---------------------------------|--------------------|--------------------------|----------------------------------------------------------------------|---------------------------------------------------------------------------|
| Chlorobenzene                   | ![Chemical structure](image) | Oxidative dechlorination | Phlebia acanthocystis, Trametes versicolor                           | Marco-Urrea et al. (2009), Bosso & Cristinzio (2014), Xiao & Kondo (2020) |
| Triazine herbicides             | ![Chemical structure](image) | N-dealkylation or N-deamination | Pleurotus pulmonarius, Phanerochaete chrysosporium                    | Masis-Mora et al. (2019)                                                  |
| Phenoxy herbicides              | ![Chemical structure](image) | Hydroxylation and dechlorination | Mortierella sp, Trichoderma viride, Trichoderma koningii, Rhizopus stolonifer | Nakagawa et al. (2006), Bhosle & Thore (2016)                              |
| Polychlorinated dibenzodioxins  | ![Chemical structure](image) | Deoxygenation            | Panellus stypticus, P. lindtneri, Phlebia sp.                        | Sato et al. (2002), Kamei & Kondo (2005), Kamei et al. (2005), Xiao et al. (2011) |
| Polychlorinated biphenyls       | ![Chemical structure](image) | Ring fission, hydroxylation and methoxylation | Phanerochaete chrysosporium, Phlebia brevispora, Pleurotus ostreatus | Yadav et al. (1995), Kamei et al. (2006), Sredlova et al. (2020) |
| Chlorinated alkanes and alkenes | ![Chemical structure](image) | Reductive dechlorination | Trametes versicolor, Irpex lacteus, Ganoderma lucidum                | Marco-Urrea et al. (2008), Tabernacka (2014), Torres-Farrada et al. (2019) |
| Chlorinated insecticides        | ![Chemical structure](image) | Hydroxylation            | Phanerochaete chrysosporium, Trametes hirsuta, Phanerochaete sordida, Pleurotus ostreatus | Mougin et al. (1996), Purnomo et al. (2017), Srivastava et al. (2019) |
| Chlorinated medicaments and personal care products | ![Chemical structure](image) | Hydroxylation            | Trametes versicolor, Pleurotus ostreatus                             | Marco-Urrea et al. (2010), Palli et al. (2017), Cruz del Alamo et al. (2020) |
that varies from hundreds of micrometers to millimeters (Espinosa-Ortiz et al. 2016). Pellets produce a bioaccumulation effect, in which they first adsorb the pollutant and then degrade it (Wang et al. 2019). According to the same authors, the growth and morphology of pellets depend on the genetic characteristics of the fungus, size of the inoculum and incubation conditions, composition of the medium, physicochemical parameters (temperature, pH), and culture conditions (hydrodynamics). Most studies involving pellets are carried out at a bench scale and in a batch regimen (Mir-Tutusaus et al. 2014; Bosso et al. 2015; Dong et al. 2017; Pernyeszi et al. 2018; Olicon-Hernandez et al. 2019). In this sense, the greatest challenge is the enhancement of pellet inoculation in continuous processes. After some time, the pellets tend to shear, and reactor maintenance strategies must be applied to prevent a reduction in the pollutant removal efficiency. Espinosa-Ortiz et al. (2016) reviewed the main configurations of bioreactors using pellets in continuous processes for various pollutants, demonstrating that airlift-type reactors present the best performances, since they keep the format of the pellets in continuous and long-duration processes. Research with the application of pellets in large-scale bioreactors to remove organochlorines is still limited. This configuration relies on the stable growth of the pellets under long detention periods and adaptation to non-sterile environments. In such environments, the competition with autochthonous microorganisms for substrate promotes a reduction in the pollutant degradation efficiency (Hai et al. 2013; Svobodova et al. 2016; Badia-Fabregat et al. 2017). In the form of pellets, auto-immobilisation allows a larger concentration of fungal biomass in the reactor, inhibiting bacterial growth (Mir-Tutusaus et al. 2018a, 2018b). Pellets of the fungus Pleurotus ostreatus presented a high triclosan removal rate in a trickle-bed reactor (TBR) inoculated in domestic effluent. After 48 hours of treatment, removal of over 70% of the initial triclosan concentration was observed (Kresinova et al. 2018). A decrease in the fungus/bacteria ratio was found over seven days. However, the degradation efficiency was not affected by the autochthonous microorganisms present in the effluent. A similar result was pointed out by Sredlova et al. (2020); phospholipid fatty acid analysis showed that Pleurotus ostreatus degrade polychlorinated biphenyls even in the presence of living fungal biomass and bacteria, throughout the experiment.

Another essential aspect of pellet growth is aeration. Badia-Fabregat et al. (2015), pointed out that aeration is an essential operational aspect, economically impacting continuous processes. In reactors inoculated with fungal pellets, the morphology, and especially the pellet size, directly affects the transfer of mass and oxygen (Garcia-Reyes et al. 2017). The production of inocula for the large-scale formation of pellets for bioremediation purposes requires cheap and straightforward methodologies. Mir-Tutusaus et al. (2018a, 2018b) developed a new inoculation method that allows the large-scale production of pellets for continuous processes. The system works through the homogenization of the pellets and culture medium produced in an initial batch, and then recirculated for the posterior batch. The production takes place continuously, and the new pellets are pumped to a digester and then to the treatment reactor.

Notably, the removal efficiency may be higher in non-sterile environments due to the consortium between fungi and bacteria. On the other hand, the presence of autochthonous microorganisms increases the competition for the survival of the fungi (Mir-Tutusaus et al. 2019). A good strategy for a large-scale application requires establishing a balance between these two aspects. Recent studies have focused on using this interspecies relationship between fungi and bacteria to increase the degradation capacity and immobilization.

**Self-immobilized biomixtures**

Because autochthonous microorganisms are familiar with reactors that operate under real wastewater conditions (Badia-Fabregat et al. 2017; Cruz del Álamo et al. 2020), a consortium between fungi and other microorganisms has shown to be an excellent alternative to treatment improvement. In this case, besides enabling the application in non-sterile environments, the interspecies mechanisms produced by the consortium may increase the metabolic capacity for biodegradation of more complex compounds, such as organochlorines. As described earlier, the stability of the cells, the increase in surface area, and the porous structure of fungal pellets make them great immobilizers of other microorganisms such as bacteria, forming a kind of biomixture. The adhesion of bacteria to the pellets occurs through the simultaneous cultivation of the pellets and bacteria (Wang et al. 2019), which occurs in three stages (Figure 1).

Previous studies have already focused on the specific biosorption and biodegradation mechanisms of organochlorines by combining fungi and bacteria (Hai et al. 2012; Knudsen et al. 2013; Elleegaard-Jensen et al. 2014). These studies emphasize that the presence of fungal pellets contributes to bacteria dispersion due to the fact that hyphae act as a transport for bacteria. Therefore, the emerging
trend is the immobilization of biomixtures for further scale-up and application in continuous processes.

Recently, a removal mechanism for the organochlorinated herbicide atrazine was proposed using a biomixture of pellets of Aspergillus niger Y3 and Arthrobacter sp. ZXY-2 (Yu et al. 2020). The authors verified that the porous structure of the pellets, along with the presence of EPS, allowed the adsorption of ZXY-2 for the formation of the biomixture. After its formation, the immobilization of atrazine by the biomixture was observed and its posterior biodegradation by the ZXY-2 strain present in the pellets. Since biomixtures have demonstrated greater degradation capacity due to the synergistic effect they promote, futures studies should investigate materials that enable the connection between pellets and bacteria, as well as favorable environmental conditions for immobilization. Considering that pellets are the result of mycelium agglomeration and entanglement of hyphae, porous material would be preferable.

The morphology of the pellets allows the use of microorganisms with different metabolic activities. The surface is the most metabolically active region, with greater susceptibility to aerobic microorganisms.

The proximity to the pellet core increases the propensity for anaerobic conditions. This makes it possible to combine different types of microorganisms to degrade complex pollutants. However, studies evaluating these possibilities are still scarce.

**Immobilization onto carriers**

The current focus of the studies on immobilization is to guarantee the survival and activity of the fungi in the continuous processes. To achieve this goal, immobilization through support has been addressed either through the use of low-cost materials or residues stemming from different production processes. The combination of more than one material, or materials, and microorganisms has also been employed, increasing the adsorption and biodegradation capacity (Yu et al. 2020).

Physical characterization technologies, such as scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR), are used to understand the role of each material in the organochlorine compound removal process. Inert materials such as foams, clay particles, magnetic nanoparticles, and non-inert materials such as biochar and lignocellulosic residues, have been employed as support materials. Polyurethane foam is one of the synthetic materials mostly used in continuous processes because, under turbulent conditions, it facilitates homogeneous and persistent colonization of the biomass (Spina et al. 2012). Motivated by the desire to find proper immobilization conditions and study the adsorption of diclofenac to polyurethane foam, as well as biodegradation under flow conditions, Stenholm et al. (2019) conducted semi-continuous flow bioreactor experiments using polyurethane foam as a support material for Trametes versicolor immobilization. The authors concluded that the immobilization resulted in a laccase activity twice as large as lower-scale experiments with dispersed biomass, maintaining similar reactor volume proportions, number of pellets, and foam amount. The same material was used for the immobilization of ascomycete Penicillium oxalicum in order to remove diclofenac in a bench reactor, with the primary removal mechanism observed being biosorption. The SEM technology was used to characterize the pellets and the support
material, with no indication of EPS production, demonstrating that the immobilization occurred through physical and mechanical mechanisms (Olcon-Hernandez et al. 2019). The prevalent immobilization mechanism generally depends on the strain and, mainly, on the metabolic differences between the different fungi phyla. Although ascomycetes without ligninolytic enzymes are little explored, previous studies have shown that CYP enzymes present in these fungi are crucial for elimination of organochlorines compounds (Chefetz et al. 2019; Olcon-Hernandez et al. 2019). The exploration of more ascomycete species can assist in defining which specific CYP450 enzymes are involved in the degradation of organochlorine compounds.

The Ascomycete Pichia kudriavzevii was immobilized using two methods: encapsulation on a sodium alginate and polyvinyl alcohol matrix, and biofilm formation on clay particles. The ability of immobilized cultures to remove atrazine was evaluated continuously in a packed bed column. The clay particles proved to be an effective and economically viable support medium. Its morphological characteristics, size, irregular shape, porosity, and mechanical resistance allowed adequate immobilization and high atrazine removal efficiency (Abigail & Das 2015). Both immobilization techniques protected yeast from atrazine, in addition to allowing degradation of the contaminant in high concentrations. These results demonstrate the versatility that immobilization adds to the degradation of organochlorine compounds, which are mostly toxic. Moreover, immobilization prevents cell leakage during operation in continuous process. This is essential to maintain the intracellular enzyme activity in the first biodegradation stages of many organochlorine compounds’ biodegradation (Xiao & Kondo 2019; Mtibaa et al. 2020).

Similarly, a bionanomaterial was built through the encapsulation of Saccharomyces cerevisiae and magnetic Fe₃O₄ nanoparticles in a sodium alginate and polyvinyl alcohol matrix for atrazine removal. Biochar was added to the Fe₃O₄ nanoparticles to increase the adsorption capacity of the material. FTIR analysis demonstrated the presence of functional groups (C=O, C-OH), suggesting that the addition of biochar could provide more active sites for contaminant adsorption. Removal of approximately 95% was achieved under optimal conditions, and biodegradation proved to be the dominant atrazine removal mechanism, given that the herbicide was consumed by Saccharomyces cerevisiae as the only carbon source (Wu et al. 2018). The authors observed drastic changes in removal efficiency as the pH value decreased, which is a disadvantage compared to immobilization through the formation of biofilm in the support medium. Interestingly, it has been noted that fungi added in the form of biofilm are less susceptible to changes in environmental conditions (Abigail & Das 2015; Cruz del Álamo et al. 2020). Thus, the use of biofilm is a simpler and useful alternative in real treatment conditions.

Biochar and nanoparticles have already been used as support materials to remove metals, dyes, and other organic pollutants (Dash et al. 2009; Xu et al. 2012; Xu et al. 2015; Jack et al. 2019; Li et al. 2020). Figure 2 summarises the distribution of different uses of immobilized fungi. In particular, nearly 38% of studies have described its use for the removal of dyes from wastewater, and 20% of studies have utilised it for the removal of organochlorine compounds. The number is still scarce but indicates an increase over the last years. Immobilized fungi have also been applied to the removal of polymeric hydrocarbons and polycyclic aromatic hydrocarbon (17%) in food industrial effluent (12.6%), drugs and endocrine-disrupting chemicals (6.7%) and metals (5.9%), which illustrates the broad applicability of immobilized fungi in environmental bioremediation.

Recently, Yu et al. 2020 applied biochar made of maize straw to intensify the immobilization of the biomixture of Aspergillus niger Y3 and Arthrobacter sp. ZXY-2 and achieved significant atrazine removal in bench experiments. The authors reported that the addition of biochar increased the operational stability of the biomixture in terms of biodegradation. It also promoted greater pellet strength and, consequently, better survival conditions to Arthrobacter sp. ZXY-2. The SEM and FTIR techniques revealed that the addition of biochar reinforced the connection between pellets and ZXY-2 through the reduction of the repulsion among their ions (Yu et al. 2020). Zhang et al. (2020) used magnetized chitosan nanocomposites for the

![Figure 2](image-url) | Pollutant removal by mycelial cell immobilized into carriers, according to the type of pollutant (data from Web of Science™ core collection, from 2003 to 2020, Topic – biodegradation AND immobilized fungi, sample number – 133).
immobilization of *Aspergillus sydowii*. This material was evaluated to remove the organochlorinated insecticide metrifonate. The authors observed a rise in the removal of the insecticide as the nanocomposite dosage increased. The result was attributed to the porous structure of the material, which provided a large number of active sites and better conditions for the immobilization of the strain. FTIR revealed that the stretching of the C=O bond would possibly be related to the intermediaries of the metrifonate degradation (Zhang et al. 2020).

Ehlers & Rose (2005) identified some advantages using non-inert materials for the degradation of 2,4,6-trichlorophenol. Furthermore, scanning electron microscopy demonstrated that strains of *Phanerochaete chrysosporium*, *Trametes versicolor* and *Lentinula edodes*, which were inoculated in a trickle-bed reactor, used wood scraps not only as a substrate for immobilization but also as a carbon source. Apart from the benefit of immobilization, the authors identified that the bacteria present in the reactor did not use the carbon source, thus eliminating the substrate competition. Elgueta et al. (2016) used different sawdust, starch, cornmeal, and linseed concentrations to form three pelletized support media. With the highest linseed concentration (15%), the first system presented the highest atrazine removal by the fungus *Anthracophyllum discolor*. The authors identified that this system also provided more significant MnP activity. This statement may be explained by linseed composition, which is approximately 47% cellulose (Bekhit et al. 2018). Still, according to Elgueta et al. (2016), part of the cellulose present in the linseed may be biodegraded by white-rot fungi, encouraging expressive growth and increasing the production of ligninolytic enzymes. For this reason, the ability of some fungi to degrade cellulose, lignin, and hemicellulose, especially white-rot fungi, makes it so that some works use non-inert materials as a form of immobilization. More recently, disks made of polypropylene inserted into a rotating biological contactor (RBC) (commonly used in the primary treatment of residual waters) were employed for the immobilization of fungus *Trametes versicolor* and the removal of organochlorinated drugs (Cruz del Álamo et al. 2020). A 10 L reactor was equipped with five rotating disks, providing a total surface area of 0.71 m² for the immobilization of the fungus. The bioreactor inoculated with fungal mycelium initially operated in a batch-fed mode for 30 days. After this period, the reactor started operating continuously, being fed by the real wastewater of two different treatment plants. In this case, the organochlorine compounds were added to the wastewater. The operating conditions (no fungal biomass replacement, no addition of an alternative glucose source, and a hydraulic retention time of 1 day) adjusted well for continuous application, resulting in total organic carbon removals between 70 and 75%. Although the authors have identified the proliferation of bacteria, biological tests using bactericides and fungicides demonstrated that the fungal community played a central role in the treatment process.

Overall, this type of study must be encouraged, as it uses immobilization in continuous processes and real wastewater. Furthermore, the characterization of the fungal mycelium structure after its exposure to organochlorinated wastewater is essential. In this case, it is necessary to evaluate the compound incorporation to the mycelium and possible alterations in its physiology (Serbent et al. 2020). In general, works addressing the determination of the predominating families and genera of bacteria and fungi in reactors treating organochlorine-contaminated wastewater are concerning issues that need to be investigated.

**CONCLUSIONS AND PERSPECTIVES**

Notably, most of the chloro-organic compounds removed via mycelium immobilization belong to the group of medicaments, personal care products and pesticides. The number of studies using medicaments as target compounds is higher, particularly in applying continuous processes for their treatment. Furthermore, some studies reported the complete degradation of medicaments.

With regard to pesticides, the vast majority of studies agree that compared to free cells, there is an increase in the percentage of adsorption and efficiency when the immobilized mycelium is used. However, reported experiences are still scarce, and the most successful cases have been conducted in flasks or small bioreactors with controlled conditions. Because of their residuality and persistence, it is also crucial that new studies determine the toxicity of products, which can be even more toxic than the parent compound.

The immobilisation gives characteristics to the fungi that enhance the degradation capacity of organochlorine compounds by increasing their enzymatic activity and biosorption capacity, and lead to more robust operations in continuous mode. The advantages of using immobilized fungi are corroborated independently of the method or material used for the immobilisation. Nevertheless, only a few studies specify the mechanisms of immobilization, biosorption and degradation through characterization technologies. In this sense, studies comparing the material-
fungi system before and after the removal of chloro-organic pollutants should be encouraged so as to elucidate the function of each component and evaluate the desorption phenomena on the mycelium and the different carriers.

Even though fungal bioconversion of chloro-organic compounds has been mainly associated with *Trametes* and *Aspergillus*, immobilization technics, such as entrapment, have made it feasible to explore new species in recent years, including unicellular fungi *Pichia kudriavzevii* and *Saccharomyces cerevisiae*. This approach may help determine the right organism with the appropriate characteristics for carrying out the desired organochlorine remediation. Moreover, preliminary and recent studies suggest the potential of biomixtures to strengthen the bioremediation capacity of organochlorine-degrading microorganisms. However, further efforts are necessary to comprehend the interspecies interactions between fungal pellets and bacteria, particularly how to enhance the bacterial fixability by pellets in continuous mode. In this regard, the exploration and application of new substances that can provide more binding sites for the pellets are essential.

In this perspective, the organochlorine removal mechanisms by immobilized fungi require additional investigations. This includes studies on new fungi species and different materials to elucidate the genetic and physiologic variations in the mycelium, according to the material and immobilization method used. Therefore, these researches will contribute to developing cost-effective and efficient techniques to remediate organochlorines in real wastewaters.

Further experimental investigations are needed to improve the fungi’s recyclability and its application as a circular economy technology. In summary, we acknowledge that the topic addressed in this paper is still an ongoing subject for future research.

**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

**REFERENCES**

Abigail, E. A. M. & Das, N. 2015 Removal of atrazine from aqueous environment using immobilized *Pichia kudriavzevii* Atz-EN-01 by two different methods. *International Biodeterioration & Biodegradation* 104, 53–58.

Antecka, A., Bizukojc, M. & Ledakowicz, S. 2016 Modern morphological engineering techniques for improving productivity of filamentous fungi in submerged cultures. *World Journal of Microbiology & Biotechnology* 32 (12), 193.

Badia-Fabregat, M., Lucas, D., Gros, M., Rodriguez-Mozaz, S., Barcelo, D., Caminal, G. & Vicent, T. 2015 Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. *Journal of Hazardous Materials* 283, 663–671.

Badia-Fabregat, M., Lucas, D., Tuomivirta, T., Fritze, H., Pennanen, T., Rodriguez-Mozaz, S., Barcelo, D., Caminal, G. & Vicent, T. 2017 Study of the effect of the bacterial and fungal communities present in real wastewater effluents on the performance of fungal treatments. *Science of the Total Environment* 579, 366–377.

Barber, E. A., Liu, Z. Y. & Smith, S. R. 2020 Organic contaminant biodegradation by oxidoreductase enzymes in wastewater treatment. *Microorganisms* 8 (1), 122.

Behloul, M., Lounici, H., Abdle, N., Drouiche, N. & Mameri, N. 2017 Adsorption study of metribuzin pesticide on fungus *Pleurotus mutilus*. *International Biodeterioration & Biodegradation* 119, 687–695.

Bekhit, A. E. A., Shavandi, A., Jodjaja, T., Birch, J., Teh, S., Ahmed, I. A. M., Al-Juhaimi, F. Y., Saeedi, P. & Bekhit, A. A. 2018 Flaxseed: composition, detoxification, utilization, and opportunities. *Biocatalysis and Agricultural Biotechnology* 13, 129–152.

Bhosle, N. P. & Thore, A. S. 2016 Biodegradation of the herbicide 2,4-D by some fungi. *American-Eurasian Journal of Agricultural & Environmental Sciences* 16 (10), 1666–1671.

Bilal, M., Asgher, M., Iqbal, H. M. N., Hu, H. B. & Zhang, X. H. 2017 Bio-based degradation of emerging endocrine-disrupting and dye-based pollutants using cross-linked enzyme aggregates. *Environmental Science and Pollution Research* 24 (8), 7035–7041.

Bosso, L. & Cristinzio, G. 2014 A comprehensive overview of bacteria and fungi used for pentachlorophenol biodegradation. *Reviews in Environmental Science and Bio-Technology* 13 (4), 387–427.

Bosso, L., Lacatena, F., Cristinzio, G., Cea, M., Diez, M. C. & Rubilar, O. 2015 Biosorption of pentachlorophenol by *Anthracophyllum discolor* in the form of live fungal pellets. *New Biotechnology* 32 (1), 21–25.

Cecchi, G., Vagge, G., Cutroneo, L., Greco, G., Di Piazza, S., Faga, M., Zotti, M. & Capello, M. 2019 Fungi as potential tool for polluted port sediment remediation. *Environmental Science and Pollution Research* 26 (35), 35602–35609.

Chefetz, B., Marom, R., Salton, O., Oliferovsky, M., Mordehay, V., Ben-Ari, J. & Hadar, Y. 2019 Transformation of lamotrigine by white-rot fungus *Pleurotus ostreatus*. *Environmental Pollution* 250, 546–553.

Chun, S. C., Muthu, M., Hasan, N., Tasneem, S. & Gopal, J. 2019 Mycoremediation of PCBs by *Pleurotus ostreatus*: possibilities and prospects. *Applied Sciences-Basel* 9 (19), 4185.

Coelho-Moreira, J. D., Brugnari, T., Sa-Nakanishi, A. B., Castoldi, R., de Souza, C. G. M., Bracht, A. & Peralta, R. M. 2018
Evaluation of diuron tolerance and biotransformation by the white-rot fungus *Ganoderma lucidum*. Fungal Biology 122 (6), 471–478.

Cruz del Álamo, A., Pariente, M. I., Martínez, F. & Molina, R. 2020 *Trametes versicolor* immobilized on rotting biological contactors as alternative biological treatment for the removal of emerging concern micropollutants. Water Research 170, 115313.

Dalecka, B., Oskarsson, C., Juhna, T. & Rajaraao, G. K. 2020 Isolation of fungal strains from municipal wastewater for the removal of pharmaceutical substances. Water 12 (2), 524.

Dash, R. R., Balomajumder, C. & Kumar, A. 2009 Removal of metal cyanides from aqueous solutions by suspended and immobilized cells of *Rhizopus oryzae* (MTCC 2541). *Engineering in Life Sciences* 9 (1), 53–59.

Dong, Y. H., Li, L., Hu, X. M. & Wu, C. H. 2017 Optimization of o-chlorophenol biodegradation by combined mycelial pellets using response surface methodology. *Water Air and Soil Pollution* 228 (11), 431.

Ehlers, G. A. & Rose, P. D. 2005 Immobilized white-rot fungal biodegradation of phenol and chlorinated phenol in trickling packed-bed reactors by employing sequencing batch operation. *Bioresource Technology* 96 (11), 1264–1275.

Elgueta, S., Santos, C., Lima, N. & Diez, M. C. 2016 Immobilization of the white-rot fungus *Anthracophyllum discolor* to degrade the herbicide atrazine. *AMB Express* 6, 104.

Ellegaard-Jensen, L., Knudsen, B. E., Johansen, A., Albers, C. N., Aamand, J. & Rosendahl, S. 2014 Fungal-bacterial consortia increase diuron degradation in water-unsaturated systems. *Science of the Total Environment* 466, 699–705.

Espinoza-Ortiz, E. J., Rene, E. R., Pakshirajan, K., van Hullebusch, E. D. & Lens, P. N. L. 2016 Fungal pelleted reactors in wastewater treatment: applications and perspectives. *Chemical Engineering Journal* 283, 553–571.

Forgacs, E., Cserhati, T. & Oros, G. 2004 Removal of synthetic dyes from wastewaters: a review. *Environment International* 30 (7), 953–971.

García-Reyes, M., Beltran-Hernandez, R. I., Vazquez-Rodriguez, G. A., Coronel-Olivares, C., Medina-Moreno, S. A., Juarez-Santillan, L. F. & Lucho-Constantino, C. A. 2017 Formation, morphology and biotechnological applications of filamentous fungal pellets: a review. *Revista Mexicana De Ingenieria Quimica* 16 (3), 703–720.

García-Zamora, J. L., Leon-Aguirre, K., Quiroz-Morales, R., Parra-Saldivar, R., Gomez-Patino, M. B., Arrieta-Baez, D., Rebollar-Perez, G. & Torres, E. 2018 Chloroperoxidase-mediated halogenation of selected pharmaceutical micropollutants. *Catalysts* 8 (1), 32.

Gibbs, P. A., Seviour, R. J. & Schmid, P. 2000 Growth of filamentous fungi in submerged culture: problems and possible solutions. *Critical Reviews in Biotechnology* 20 (1), 17–48.

Gotovtsev, P. M., Yuzbasheva, E. Y., Gorin, K. V., Butylin, V. V., Badranova, G. U., Perkovskaya, N. I., Mostova, E. B., Namsaraev, Z. B., Rudneva, N. I., Komova, A. V., Vasilov, R. G. & Sineokii, S. P. 2015 Immobilization of microbial cells for biotechnological production: modern solutions and promising technologies. *Applied Biochemistry and Microbiology* 51 (8), 792–803.

Hai, F. I., Modin, O., Yamamoto, K., Fukushima, K., Nakajima, F. & Nghiem, L. D. 2012 Pesticide removal by a mixed culture of bacteria and white-rot fungi. *Journal of the Taiwan Institute of Chemical Engineers* 43 (3), 459–462.

Hai, F. I., Yamamoto, K., Nakajima, F., Fukushima, K., Nghiem, L. D., Price, W. E. & Jin, B. 2013 Degradation of azo dye acid orange 7 in a membrane bioreactor by pellets and attached growth of *Coriolus versicolor*. *Bioresource Technology* 141, 29–34.

Harms, H., Schlosser, D. & Wick, L. Y. 2011 Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology* 9 (3), 177–192.

Haugland, J. O., Kinney, K. A., Johnson Jr, W. H., Camino, M. M. A., Whitman, C. P. & Lawler, D. F. 2009 Laccase removal of 2-chlorophenol and sulframethoxazole in municipal wastewater. *Water Environment Research* 91 (4), 281–291.

Hussain, R., Ahmed, M., Khan, T. A. & Akhter, Y. 2020 Fungal P450 monoxygenases-the diversity in catalysis and their promising roles in biocontrol activity. *Applied Microbiology and Biotechnology* 104 (3), 989–999.

Jack, J., Huggins, T. M., Huang, Y. P., Fang, Y. F. & Ren, Z. J. 2019 Production of magnetic biochar from waste-derived fungal biomass for phosphorus removal and recovery. *Journal of Cleaner Production* 224, 100–106.

Jin, X. T., Yu, X. Y., Zhu, G. Y., Zheng, Z. T., Feng, F. Y. & Zhang, Z. Y. 2016 Conditions optimizing and application of laccase-mediator system (LMS) for the laccase-catalyzed pesticide degradation. *Scientific Reports* 6, 35787.

Kamei, I. & Kondo, R. 2005 Biotransformation of dichloro-, trichloro-, and tetrachlorodibenzo-p-dioxin by the white-rot fungus *Phlebia lindtneri*. *Applied Microbiology and Biotechnology* 68 (4), 560–566.

Kamei, I., Suhara, H. & Kondo, R. 2005 Phylogenetical approach to isolation of white-rot fungi capable of degrading polychlorinated dibenzo-p-dioxin. *Applied Microbiology and Biotechnology* 69 (3), 358–366.

Kamei, I., Sonoki, S., Haraguchi, K. & Kondo, R. 2006 Fungal bioconversion of toxic polychlorinated biphenyls by white-rot fungus *Phlebia brevispora*. *Applied Microbiology and Biotechnology* 73 (4), 932–940.

Kaur, H., Kapoor, S. & Kaur, G. 2016 Application of ligninolytic potentials of a white-rot fungus *Ganoderma lucidum* for degradation of lindane. *Environmental Monitoring and Assessment* 188 (10), 588.

Knudsen, B. E., Ellegaard-Jensen, L., Albers, C. N., Rosendahl, S. & Aamand, J. 2013 Fungal hyphae stimulate bacterial degradation of 2,6-dichlorobenzamide (BAM). *Environmental Pollution* 181, 122–127.

Kresinova, Z., Linhartova, L., Filipova, A., Ezechias, M., Masin, P. & Cajthaml, T. 2018 Biodegradation of endocrine disruptors in urban wastewater using *Pleurotus ostreatus* bioreactor. *New Biotechnology* 45, 53–61.
Wittmann, C. 2015 Characterization and control of fungal morphology for improved production performance in biotechnology. *Journal of Biotechnology* **163** (2), 112–123.

Kulshreshtha, S. 2019 Removal of pollutants using spent mushrooms substrates. *Environmental Chemistry Letters* **17** (2), 835–847.

Kumar, D. & Pannu, R. 2018 Perspectives of lindane (γ-hexachlorocyclohexane) biodegradation from the environment: a review. *Bioresources and Bioprocessing* **5**, 29.

Le, T. T., Murugesan, K., Lee, C. S., Vu, C. H., Chang, Y. S. & Jeon, J. R. 2016 Degradation of synthetic pollutants in real wastewater using laccase encapsulated in core-shell magnetic copper alginate beads. *Bioresource Technology* **216**, 203–210.

Li, H. Y., Liu, L. X., Cui, J. G., Cui, J. L., Wang, F. & Zhang, F. 2020 High-efficiency adsorption and regeneration of methylene blue and aniline onto activated carbon from waste edible fungus residue and its possible mechanism. *RSC Advances* **10** (24), 14262–14273.

Liu, Y., Wu, Y. Y., Zhang, Y., Yang, X. L., Yang, E., Xu, H. N., Yang, Q. L., Chagán, I., Cui, X. M., Chen, W. M. & Yan, J. P. 2019 Lignin degradation potential and draft genome sequence of *Trametes trogii* s0301. *Biotechnology for Biofuels* **12**(1), 256.

Mani, D. & Kumar, C. 2014 Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. *International Journal of Environmental Science and Technology* **11** (3), 843–872.

Marco-Urrea, E., Gabarrell, X., Camelín, G., Vicent, T. & Reddy, C. A. 2008 Aerobic degradation by white-rot fungi of trichloroethylene (TCE) and mixtures of TCE and perchloroethylene (PCE). *Journal of Chemical Technology and Biotechnology* **83** (9), 1190–1196.

Marco-Urrea, E., Perez-Trujillo, M., Camelín, G. & Vicent, T. 2009 Dechlorination of 1,2,3- and 1,2,4-trichlorobenzene by the white-rot fungus *Trametes versicolor*. *Journal of Hazardous Materials* **166** (2–3), 1141–1147.

Marco-Urrea, E., Perez-Trujillo, M., Cruz-Morato, C., Camelín, G. & Vicent, T. 2010 Degradation of the drug sodium diclofenac by *Trametes versicolor* pellets and identification of some intermediates by NMR. *Journal of Hazardous Materials* **176** (1–3), 836–842.

Masis-Mora, M., Lizardo-Fallas, V., Tortella, G., Beita-Sandi, W. & Rodriguez-Rodriguez, C. E. 2019 Removal of triazines, triazoles and organophosphates in biomixtures and intermediates by NMR. *Journal of Biotechnology* **842**. 847.

Mir-Tutusaus, J. A., Masis-Mora, M., Barcelo, D., Sarra, M., Camelín, G., Vicent, T. & Rodriguez-Rodriguez, C. E. 2014 Degradation of selected agrochemicals by the white rot fungus *Trametes versicolor*. *Science of the Total Environment* **500**, 235–242.

Mir-Tutusaus, J. A., Baccar, R., Camelín, G. & Sarra, M. 2018a Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal? a review. *Water Research* **138**, 157–151.

Mir-Tutusaus, J. A., Camelín, G. & Sarra, M. 2018b Influence of process variables in a continuous treatment of non-sterile hospital wastewater by *Trametes versicolor* and novel method for inoculum production. *Journal of Environmental Management* **212**, 415–423.

Müttø, R., Ezzanad, A., Aranda, E., Pozo, C., Ghariani, B., Moraga, J., Nasri, M., Cantoral, J. M., Garrido, C. & Mechichi, T. 2020 Biodegradation and toxicity reduction of nonylphenol, 4-tert-octylphenol and 2,4-dichlorophenol by the ascomycetous fungus *Thielavia sp* HJ22: identification of fungal metabolites and proposal of a putative pathway. *Science of the Total Environment* **708**, 135129.

Nakagawa, A., Osawa, S., Hirata, T., Yamagishi, Y., Hosoda, J. & Horikoshi, T. 2006 2,4-dichlorophenol degradation by the soil fungus *Mortierella sp*. *Bioscience Biotechnology and Biochemistry* **70**(2), 525–527.

Olicon-Hernandez, D. R., Camacho-Morales, R. L., Pozo, C., Gonzalez-Lopez, J. & Aranda, E. 2009 Evaluation of diclofenac biodegradation by the ascomycete fungus *Penicillium oxalicum* at flask and bench bioreactor scales. *Science of the Total Environment* **662**, 607–614.

Palli, L., Castellet-Rovira, F., Perez-Trujillo, M., Caniani, D., Sarra-Adrogue, M. & Gori, R. 2017 Preliminary evaluation of *Pleurotus ostreatus* for the removal of selected pharmaceuticals from hospital wastewater. *Biotechnology Progress* **33** (6), 1529–1537.

Pernyész, T., Farkas, V., Felinger, A., Boros, B. & Dekany, I. 2018 Use of non-living lyophilized *Phanerochaete chrysosporium* cultivated in various media for phenol removal. *Environmental Science and Pollution Research* **25** (9), 8530–8562.

Pulicharla, R., Das, R. K., Brar, S. K., Drögü, P. & Surampalli, R. Y. 2018 Degradation kinetics of chlortetracycline in wastewater using ultrasound assisted laccase. *Chemical Engineering Journal* **347**, 828–835.

Purnomo, A. S., Nawfa, R., Martak, F., Shimizu, K. & Kamei, I. 2017 Biodegradation of Aldrin and Dieldrin by the White-Rot Fungus *Pleurotus ostreatus*. *Current Microbiology* **74**(7), 889–889.
Pylypchuk, I. V., Kessler, V. G. & Seisenbaeva, G. A. 2018
Simultaneous removal of acetalaminophen, diclofenac, and Cd (II) by Trametes versicolor laccase immobilized on Fe3O4/SiO2-DTPA hybrid nanocomposites. ACS Sustainable Chemistry & Engineering 6 (8), 9979–9989.

Ravelo, D. C., Corral, O. L., Gonzalez-Martinez, I., Cupul, W. C. & Nava, C. O. R. 2020 Evaluation of bezafibrate, gemfibrozil, indomethacin, sulfamethoxazole, and diclofenac removal by ligninolytic enzymes. Preparative Biochemistry & Biotechnology 50 (6), 592–597.

Sarker, A., Lee, S. H., Kwak, S. Y., Nandi, R. & Kim, J. E. 2020
Comparative catalytic degradation of a metabolite 3,5-dichloroaniline derived from dicarboximide fungicide by laccase and MnO2 mediators. Ecotoxicology and Environmental Safety 196, 110561.

Sato, A., Watanabe, T., Watanabe, Y., Harazono, K. & Fukatsu, T. 2014 Biological treatment of groundwater polluted with chlorinated ethene. Ochrona Srodowiska 36 (1), 9–13.

Torres-Farrada, G., Manzano-Leon, A. M., Rineau, F., Leal, M. R., Thijs, S., Jambon, I., Put, J., Czech, J., Rivera, G. G., Carlee, R. & Vangronsveld, J. 2019 Biodegradation of polycyclic aromatic hydrocarbons by native Ganoderma sp. strains: identification of metabolites and proposed degradation pathways. Applied Microbiology and Biotechnology 103 (17), 7203–7215.

UNEP (United Nations Environment Programme) 2017
Second global monitoring report: the eight meeting of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants. Geneva. Available from: http://chm.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP8/tabid/5309/ctl/Download/mid/16170/Default.aspx?Id=85&ObjID=23557 (accessed 22 June 2020).

Vidal-Limon, A., Suarez, P. C. G., Arellano-Garcia, E., Contreras, O. E. & Aguila, S. A. 2018 Enhanced degradation of pesticide dichlorophen by laccase immobilized on nanoporous materials: a cytotoxic and molecular simulation investigation. Bioconjugate Chemistry 29 (4), 1073–1080.

Wang, M. X., Zhang, Q. L. & Yao, S. J. 2015 A novel biosorbent formed of marine-derived Penicillium janthinellum mycelial pellets for removing dyes from dye-containing wastewater. Chemical Engineering Journal 259, 837–844.

Wang, H. L., Ping, L., Yu, Q. & Hui, Y. 2016 Removal of phenol in phenolic resin wastewater by a novel biomaterial: the Phanerochaete chrysosporium pellet containing chlamydospore-like cells. Applied Microbiology and Biotechnology 100 (11), 5153–5164.

Wang, L., Yu, T. M., Ma, F., Vitus, T., Bai, S. S. & Yang, J. X. 2019 Novel self-immobilized biomass mixture based on mycelium pellets for wastewater treatment: a review. Water Environment Research 91 (2), 93–100.

Wu, X., He, H., Yang, W. L., Yu, J. & Yang, C. 2018 Efficient removal of atrazine from aqueous solutions using magnetic Saccharomyces cerevisiae biomaterial. Applied Microbiology and Biotechnology 102, 7597–7610.

Xiao, P. F. & Kondo, R. 2009 Biodegradation and bioconversion of endrin by white rot fungi, Phlebia acanthocystis and Phlebia brevispora. Mycoscience 60 (4), 255–261.

Xiao, P. F. & Kondo, R. 2020 Biodegradation and biotransformation of pentachlorophenol by wood-decaying white rot fungus Phlebia acanthocystis TMIC34875. Journal of Wood Science 66 (1), 2.

Xiao, P. F., Mori, T., Kamei, I., Kiyota, H., Takagi, K. & Kondo, R. 2011 Novel metabolic pathways of organochlorine pesticides dieldrin and aldrin by the white rot fungus of the genus Phlebia. Chemosphere 85 (2), 218–224.

Xu, P. A., Zeng, G. M., Huang, D. L., Lai, C., Zhao, M. H., Wei, Z., Li, N. J., Huang, C. & Xie, G. X. 2012 Adsorption of Pb (II) by iron oxide nanoparticles immobilized Phanerochaete chrysosporium: equilibrium, kinetic, thermodynamic and mechanisms analysis. Chemical Engineering Journal 203, 423–431.

Xu, W. H., Jian, H., Liu, Y. G., Zeng, G. M., Li, X., Gu, Y. L. & Tan, X. F. 2015 Removal of chromium (VI) from aqueous
solution using mycelial pellets of *Penicillium simplicissimum* impregnated with powdered biochar. *Bioremediation Journal* **19** (4), 259–268.

Yadav, J. S., Wallace, R. E. & Reddy, C. A. 1995 Mineralization of monochlorobenzenes and dichlorobenzenes and simultaneous degradation of chloro-substituted and methyl-substituted benzenes by the white-rot fungus *phanerochaete-chrysosporium*. *Applied and Environmental Microbiology* **61** (2), 677–680.

Yesilada, O., Yildirim, S. C., Birhanli, E., Apohan, E., Asma, D. & Kuru, F. 2010 The evaluation of pre-grown mycelial pellets in decolorization of textile dyes during repeated batch process.

World *Journal of Microbiology & Biotechnology* **26** (1), 33–39.

Yu, T. M., Wang, L., Ma, F., Wang, Y. J. & Bai, S. S. 2020 A biofunctions integration microcosm: self-immobilized biochar-pellets combined with two strains of bacteria to remove atrazine in water and mechanisms. *Journal of Hazardous Materials* **384**, 121326.

Zhang, C., Chen, Z. X., Tao, Y., Ke, T., Li, S. X., Wang, P. P. & Chen, L. Z. 2020 Enhanced removal of trichlorfon and Cd(II) from aqueous solution by magnetically separable chitosan beads immobilized *Aspergillus sydowii*. *International Journal of Biological Macromolecules* **148**, 457–465.

First received 30 December 2020; accepted in revised form 26 February 2021. Available online 10 March 2021