Laccase-based biocatalytic systems application in sustainable degradation of pharmaceutically active contaminants

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Abstract

The outflow of pharmaceutically active chemicals (PhACs) exerts a negative impact on biological systems even at extremely low concentrations. For instance, enormous threats to human and aquatic species have resulted from the widespread use of antibiotics in ecosystems, which stimulate the emergence and formation of antibiotic-resistant bacterial species and associated genes. Additionally, it is challenging to eliminate these PhACs by employing conventional physicochemical water treatment techniques. Enzymatic approaches, including laccase, have been identified as a promising alternative to eliminate a broad array of PhACs from water matrices. However, their application in environmental bioremediation is hindered by several factors, including the enzyme's stability and its location in the aqueous environment. Such obstacles may be surmounted by employing laccase immobilization, which enables enhanced stability (including inactivation caused by the substrate), and thus improved catalysis. This review emphasizes the potential hazards of PhACs to aquatic organisms within the detection concentration range of ngL^{-1} to μgL^{-1}, as well as the deployment of laccase-based multifunctional biocatalytic systems for the environmentally friendly mitigation of anticancer drugs, analgesics/NSAIDs, antibiotics, antiepileptic agents, and beta blockers as micropollutants. This approach could reduce the underlying toxicological consequences. In addition, current developments, potential applications, and viewpoints have focused on computer-assisted investigations of laccase-PhACs binding at enzyme cavities and degradability prediction.

Keywords: Laccase; Pharmaceutical compounds; Biocatalysis; Enzyme immobilization; Environmental bioremediation; Ecological hazards; Toxicity

1. Introduction

A new class of organic pollutants known as pharmaceutically active compounds (PhACs) primarily consists of hormones, antibiotics, antifungal agents, antidepressants, anti-epileptics, hypoglycemic medications, analgesics, beta-blockers, and nonsteroidal anti-inflammatory medicines (NSAIDs) [1, 2]. The potential application of PhACs is widely employed in agriculture, aquaculture, animal husbandry, and human healthcare globally [1]. PhACs are not used by organisms substantially, and most of them are flushed out of the body in their parent form before being metabolized, the vast majority of these compounds end up in the environment or wastewater treatment facilities (WWTPs) [1]. The concentration of PhACs in water systems has been reported in a varying range from ng L^{-1} to µg L^{-1} [3, 4]. Their existence in aquatic ecosystems is a consequence of the direct release of WWTP effluents into water bodies [5]. These PhACs are present in both the influent and effluent wastewater, but they might also be detected in other kinds of surface water including freshwater and marine environments as well as in groundwater via effluent leachates [6]. Over the last decade, the increase in the global population has led to elevated drug use and, therefore, a significant excretion of both the drug and its metabolites [5]. The scarcity of information concerning the sources, transport, and accumulation of PhACs in aquatic ecosystems has resulted in uncertainty in associated potential risks they may exhibit during their interactions with aquatic bodies. On a global scale, these compounds are among the most critical emerging contaminants (EC) found in water sources [5]. Consequently, PhACs have devastating consequences on aquatic organisms, since exposure to them has negative reproductive effects in the early life stages of several species [7-10]. As a result, this issue requires serious consideration as a way to address it

promptly. A wide range of physical, physicochemical, and chemical methods including membrane processes, advanced oxidation processes (e.g., ozonation, UV photolysis or UV/H_2O_2) adsorption are often employed to remove pharmaceutical contamination from water [11-13]. Nevertheless, these methods have several disadvantages, including the generation of toxic by-products in advanced oxidation processes, the disposal challenges associated with concentrated waste in membrane processes, and the cost of operation, etc. [14]. For instance, the high expense of the reagents (such as H_2O_2) and energy required to generate O_3 or UV light is one major drawback of advanced oxidation processes [15]. Compared to physicochemical or other non-biological processes, biological processes particularly enzymatic remediation offer several benefits including; In addition, including advantages of environmental friendliness, high efficiency, broad substrate specificity, less toxic need less energy, and not required for any extra nutrients to operate designed experiments [14, 16].

Laccases (EC 1.10.3.2) are multicopper-containing oxidoreductases that are potent biocatalysts and have been exploited in the bioremediation of a broad range of pollutants including a wide range of PhACs [17-24]. Laccases can oxidize diverse phenolic and non-phenolic contaminant compounds employing molecular oxygen as an electron acceptor, with water as the only by-product [25, 26]. This is a clear advantage when compared to other enzymes (peroxidases or oxidases) with oxidant potential that could be used for similar functions, as both have H_2O_2 in their catalytic cycle (Fig. 1) [27]. Peroxidases have been proposed in many instances to perform this bioremediation function, but these enzymes require the use of H_2O_2 as an oxidant cosubstrate [28-31]. This means that the actual use of these enzymes in bioremediation may be hindered, as it may not be easy to add this dangerous

reagent to the environment due to its negative biological effects, although this fact used to be not considered. This may be stressed if the environment is as large as the ocean. Oxidases are other alternatives to destroy contaminant compounds [28, 32, 33]. They do not require the addition of H_2O_2 as a co-substrate (Fig.1). As laccases do, they can utilize molecular oxygen as an oxidant. However, in their catalytic cycle, the FAD-dependent oxidases produce hydrogen peroxide as a by-product [27, 34, 35]. Laccases are extensively prevalent in the environment (e.g., plants, insects, fungi, and bacteria)) [17]. Such prevalence has been caused by their multiple physiological roles. For example, within plants, they are engaged in lignin biosynthesis (polymerization from monolignols), while bacterial and fungal-derived laccase (Table 1) are most often deployed systems involved in the degradation of lignin, phenolic, and pharmaceuticals [36-38]. The catalytic reaction of laccase triggers the cleavage of each specific compound in a unique way, i.e., C-C cleavage, oxidation, or alkyl-aryl cleavage; the same pattern is utilized to break down both β-1 and β-O-4 dimers of lignin compounds [39, 40]. Laccases catalyze oxidative coupling or bond breakage of target molecules by one-electron oxidation and subsequent radical formation [41]. Such attribute of laccase may oxidize several phenolic and nonphenolic substances owing to its broad substrate specificity [42, 43]. Based on distinct spectroscopic features, the four copper atoms in a typical laccase molecule are classified as Type 1 (T1), Type 2 (T2), and binuclear Type 3 (T3) Cu sites [44]. The four copper ions in the resting enzyme are in the +2 oxidation state [44]. Plant and bacterial laccases typically have low redox potentials (e.g., 0.43 and 0.46 V for *Rhus vernicifera* and wild-type *Bacillus subtilis* CotA laccases, respectively), whilst, white-rot fungi exhibit comparatively higher redox potential (0.720–0.790 V) [41]. A rising global issue in recent years has been the presence of

ECs, which include a variety of PhACs, in both the environment and WWTPs [45, 46]. PhACs and their metabolites are usually discharged into the environment by urban agricultural discharge, hospital effluent, and treated and untreated industrial wastewater [45, 46]. Hospitals primarily discharge PhACs into the environment as a consequence of patient excretion and the improper disposal of remaining medications [46]. A schematic representation of PhACs emergence from diverse sources and their prevalence in water matrices is portrayed in Fig. 2. Several PhACs are not eliminated through WWTPs and are released into the environment either in the form of non-degraded or in metabolites [47]. Despite this, the WWTPs are not initially designed with the distinct objective of eliminating these hazardous chemicals [48]. The magnitude of toxicological hazards of such PhACs has been explained thoroughly in a separate section. For example, antibiotics such as sulfamethoxazole can induce genetic alterations and long-term effects in aquatic animals, even at low concentrations [49-51]. Conversely, analgesics such as paracetamol can increase the risk of asthma, liver damage, and kidney cancer in humans [52, 53]. Laccase-based biocatalysts are well-suited to expediting the development of environmentally friendly, sustainable, and efficient industrial deployments to mitigate the aforementioned EC from water matrices [23]. However, the affordability and effectiveness of the enzymes restrict the widespread use of laccases. Considerable efforts have been undertaken to enhance the production of substantial quantities of laccases at a low cost by the use of recombinant organisms or the identification of natural hypersecretory strains [54]. Enzyme activity and stability can be improved by employing appropriate immobilization techniques, including the use of bionanozyme methods, as discussed below [55]. The immobilization of enzymes without compromising their activity offers a potential solution to these issues and enables

reusability by simplifying the separation process and enhancing stability to a variety of environmental factors [56]. In addition to the conventional application of laccases in the degradation of ECs, they have also been employed in the computer-aided pre-screening of degradability, which involves the implementation of multivalent computational techniques that include docking, MD-simulation, DFT, protein modeling, and predicted plausible pathways for microbial degradation [57-62]. The aforementioned computer-aided methods are not only robust, but they also provide a molecular-level comprehension of the degradation process by comprehending the role of the involved amino acid residues [63].The purpose of this review article is to emphasize the potential hazards of PhACs and the potential applications of laccases in both their free and immobilized forms for the degradation of a wide range of PhACs. Additionally, the computational framework has been outlined in coupled with laccase utilization as a method to facilitate the degradation of PhACs using laccases. This approach involves prescreening-based degradability, which could aid in the exploration of the structural aspects of laccases that bind to PhACs at the cavity and associated amino-acid residues.

166 **Table 1** List of well-known laccase-producing microbial species with kinetics parameters.

Laccase producer	Substrate	K_M	V_{max}	k_{cat}	k_{cat}/K_M	Condition	Reference
Aureobasidium pullulans NAC8	Guaiacol	1.05 ± 0.12 mM	12.67 ± 0.55 μmol/ml/min	25.3 × 10^{-1} s^{-1}	2.4 × 10^3 M^{-1} s^{-1}	pH-4.5, 45 °C	[64]
Pleurotus ostreatus POXA3b	ABTS	74 μm		158 333	2.1 × 10^9	pH 3.6, 25 °C	[65]
Pleurotus ostreatus POXA3a	ABTS	70 μm		73 333	1.0 × 10^9	pH 3.6, 25 °C	[65]
Lentinus sp.	ABTS	65 μm		3382	5.2 × 10^7	pH 2.5, 70 °C	[65]
Trametes hirsuta	ABTS	41 μm		196	4.8 × 10^6	pH 5.0, 25 °C	[65]
Rigidoporus lignosus	ABTS	200 μm		1360	6.8 × 10^6	pH 3.0, 25 °C	[65]
Meripilus giganteus	ABTS	17 μm		546	3.7 × 10^7	pH 3.0, 30 °C	[65]
Agaricus bisporus	ABTS	134 μm		7885	5.9 × 10^7	pH 3.0, 65 °C	[65]
Trametes versicolor	ABTS	38 μm		26 803	6.0 × 10^8	pH 3.0, 65 °C	[65]
Tricholoma mongolicum	ABTS	2 μm		1480	6.4 × 10^8	pH 4.5, 30 °C	[65]
Yersinia enterocolitica strain 7	ABTS	675 μM	0.125 μmol/ml/min			pH 9.0 and stable at 70 °C	[66]
Aspergillus niger	ABTS					pH 4.5, 45 °C	[19]
Coriolus brevis	ABTS	0.02 mM			7.2 × 10^6	Optimal reaction pH 2.5 30–90 °C	[67]
Bacillus sp. MSK-01	Guaiacol ABTS	5.481 mM(Guaiacol), 1.624 mM (ABTS)	19.32 μM min^{-1} ml^{-1}(Guaiacol), 25.53 μM min^{-1} ml^{-1}(ABTS)			ABTS(pH 4.5), guaiacol(pH 8.0), 75 °C	[68]

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2. Routes, occurrence, and associated hazards of pharmaceutical contaminants

The disposal of pharmaceuticals and their by-products, as well as the excretion of both people and animals, bring these substances into the environment. On a worldwide level, pharmaceutical substances such as NSAIDs, anticonvulsants, β-blockers, and their metabolites have been detected in water bodies [69]. Pharmaceuticals are continuously released into the environment as a result of their extensive utilization. (i) urban domestic effluents, (ii) hospital effluents, (iii) livestock farming, which involves the excretion of pharmaceuticals and their metabolites, and (iv) pharmaceutical manufacturing serve as the most significant and extensive sources of pharmaceutical contamination in wastewater worldwide (Fig. 2) [70]. Pharmaceuticals are a substantial contributor to wastewater effluent from hospitals and health care facilities resulting from the excretion of pharmaceuticals by patients and laboratory, diagnostic, and research activities. Conventional wastewater treatment facilities are unable to adequately remove the inert chemicals and metabolites released into the environment as a result of the widespread use of pharmaceuticals [6, 71]. The prevalence of pharmaceutical substances and their metabolites in the environment is an issue of concern. Consequently, pharmaceuticals pose a concern to aquatic ecosystems and human health since they are found in water bodies in amounts ranging from ngL^{-1} to μgL^{-1} [72-76]. Many organic chemicals, along with pharmaceutics, are present in surface waters owing to inefficient removal from wastewater-treatment facilities. In addition to wastewater from municipalities and hospitals, human pharmaceuticals consumption is also derived from landfill leachates, effluent from manufacturers, and the deposition of pharmaceuticals in the environment led to prolonged existence in water

bodies. A variety of negative impacts, that include genotoxic, teratogenic, reproduction-disturbing, acute cytogenotoxic, oxidative stress, endocrine disruption, growth inhibition (EC_{50}), deformations, and others, have been observed in aquatic organisms as a result of pharmaceutical contamination [70, 77-79]. Detailed toxicological hazards of pharmaceuticals on aquatic organisms have been listed in Table 2. Gutiérrez-Noya et al. [78] reported that ibuprofen induces teratogenesis, and oxidative stress, and alters embryonic development in *Cyprinus carpio*. Subsequently, at concentrations between 1.5 and 11.5 μg L^{-1}, ibuprofen was capable of inducing alterations to embryonic development, teratogenic effects, and oxidative stress in oocytes and embryos of *Cyprinus carpio*. As a conclusion, the major embryonic development abnormalities and teratogenic consequences were delayed hatching, hypopigmentation, pericardial edema, yolk deformation, and developmental delay in *Cyprinus carpio*.

Rosas-Ramírez et al. [79] studied the teratogenic effects of paracetamol, and ciprofloxacin, and their combined use in *Danio rerio* embryos. Subsequent findings indicated that paracetamol, ciprofloxacin, and their combination decreased the survival rate of embryos by as much as 75%. Furthermore, both drugs elicited morphological changes in the embryos, resulting in their mortality. The predominantly noticed defects were scoliosis, craniofacial anomalies, hypopigmentation, growth retardation, and pericardial edema. Xu et al. [80] demonstrated that naproxen induces thyroid dysfunction in zebra fish after a 60-day exposure to varying doses (0.1, 1, 10, and 100 μg L^{-1}). Subsequent studies indicated that thyroid hormone analysis revealed significantly reduced levels of both T3 and T4.

218 **Table 2.** Main identified toxicological impacts of PhACs on aquatic animals.

Pharmaceutical compounds	Class	Toxicity profile	Reference
Ofloxacin	Antibiotic	-Ecological risk -Risk to the trophic levels: fish, daphnids, and algae -Reproductive toxicity on *Caenorhabditis elegans*	[81-83]
Norfloxacin	Antibiotic	Reproductive toxicity on *Caenorhabditis elegans*	[83]
Erythromycin	Antibiotic	Antibiotic resistance	[84, 85]
Carbamazepine	Anticonvulsant	-Toxicological effects in humans and animals even at trace concentrations -Congenital malformations, neuro-developmental	[86, 87]
Metoprolol	β-blockers	Ecotoxicological impact	[88]
Metformin	Antidiabetic	Ecotoxicological risk to: Fish, Daphnia, Rotifers, Chlorella	[89]
Ibuprofen	Analgesic	Environmental risks, effects on aquatic organisms acute toxicity	[90]
Tramadol	Opioid-analgesic	-Altering evolutionary crucial behaviors in aquatic fish -Aquatic toxicity	[91, 92]
Cephalexin	Antibiotic	-Antibiotic resistance diarrhea, skin irritation, nausea, and stomach ache	[93]
Cefradine	Antibiotic	Toxic to *Vibrio fischeri* and *Daphnia Magna*	[94]
Diclofenac	NSAIDs	-Ecological risk -Toxic to vultures, aquatic organisms, higher plants, also causes serious threats to mammals	[95]
Benzodiazepine(Alprazolam, clonazepam and diazepam)	Psychotropic drug	-Environmental risk -Bioaccumulation, behavioral changes, and modulations in genes and enzymes of fishes and insects	[96]

Paracetamol	Antipyretic	-Hepatotoxic -Ecotoxicological effects on aquatic organisms	[97, 98]
Albendazole	Anthelmintic	Toxicity on aquatic and soil organisms	[99]
Tetracycline	Antibiotic	Negative impact on algal and plankton communities Human health and environmental hazards	[100, 101]

3. Structural and chemical aspects of laccases

As stated above, laccases belong to the protein superfamily known as multicopper oxidases [102-104]. Enzymes of this type exhibit catalytic activity in the oxidation of a wide variety of compounds, notably those with phenolic moieties. Furthermore, these enzymes are not only important in numerous biological processes but also relevant in the area of biotechnology [105, 106]. The catalytic cycle of laccase may be improved by employing a mediator system to degrade the specific contaminant (Fig.3). The molecular structure of laccases is characterized by a high abundance of beta components in its protein structure, distinguishing them from peroxidases [105, 107, 108]. Although laccases are structurally complicated, they are typically monomeric enzymes [105]. The Protein Databank (https://www.rcsb.org) contains a wide range of laccases structures from many sources, characterized by their crystal structures solved by different methods containing diverse structural architects [109, 110]. For instance, the laccase from *Thermus thermophilus* (PDB: 6TYR) comprised 439 amino acids in a single chain with a molecular weight of around 49.45 kDa [109, 111, 112]. Likewise, a fungal laccase, from *Cerrena maxima* origin (PDB: 2H5U), comprises 499 amino acid residues in a single chain with a molecular weight of around 55.67 kDa [109, 113]. A laccase from plant origin (*Zea mays*), PDB: 6KLJ comprises 550 amino acid residues in a single chain with a molecular weight of

63.91 kDa [109, 114, 115]. The laccase from *Drosophila erecta*, with accession no: XP_001981736.2, translated to have 677 amino acid residues with a calculated molecular weight of 77.56 kDa [116]. The laccase from *Trametes versicolor* (PDB: 1KYA) contains 499 amino acid residues distributed in four chains (A, B, C, and D) with a molecular weight of 221.72 kDa [117]. The constituent amino-acid residues of no single laccase are identical to those of others. Detailed quantitative information on protein secondary structure elements of laccases from different sources have been portrayed in Fig. 4. However, it is essential to note that the use of bacterial and fungal laccases has been primarily employed in the degradation of PhACs; however, comparison to different origin may provide the structural similarity in few aspects. A closer look at the multicopper binding site of laccase with an active site of copper binding is portrayed in Fig. 5.

4. Chemical scheme and catalytic functionalities of laccases

The catalytic mechanism of laccases has been extensively studied and is characterized by the formation of radical species. The catalytic process of laccases commences with the mono-electronic oxidation of four equivalent reducing substrates, including aromatic and aliphatic amines and phenols. This oxidation results in the formation of organic radicals, which consume molecular oxygen before being reduced to two molecules of water [118]. The catalytic machinery of laccase consists of a four-membered copper cluster, which is also involved in water formation and release, oxygen coordination, and reduction. Fungal laccases commonly distinguish between three distinct sites within the copper cluster, each of which serves a specific function in the catalytic cycle: the “blue site” or Type 1 (T1), the "normal site" (T2), the "binuclear site" (T3) [119, 120]. The chemical reaction of laccases is characterized by the involvement of a single electron (1e-) and the

sequential oxidation of four molecules of reducing substrates. Simultaneously, two oxygen atoms undergo double electron reductions ($2x2^{e-}$) to form their respective water molecules [43]. The aforementioned process is characterized by a catalytic exchange of four hydrogen ion ($4\ H^+$) equivalents [43]. The laccase reaction may be analyzed from a structural, mechanistic, and kinetic perspective. It can be conceptualized as two half-reactions that are linked by an internal electron transfer (IET) step. This process is facilitated by the presence of catalytic copper ions situated at the T1 Cu and T2 Cu/T3 Cuα/T3 Cuβ trinuclear cluster (TNC) sites [43]. The active site of laccase corresponding to substrate (Ofloxacin) binding including mediator (ABTS) binding attributes is portrayed in Fig. 6. Both possess distinct binding orientations and binding amino acid residues.

5. Laccase immobilization for enhancing catalytic potential towards pharmaceutical degradation

Compared to laccase in its free state, the immobilization of laccase may result in improved stability concerning storage, temperature, and pH. Enzyme immobilization consists of the location of the enzyme in a confined space [121]. This started as a technology intended to simplify the capture and reutilization of these biological and initially very expensive biocatalysts [122, 123]. The recycling, operational stability, and resistance to application conditions of laccases are all enhanced when the enzymes are immobilized [124]. Some examples of immobilization methods encompass entrapment, adsorption, covalent binding, self-immobilization, and so on has portrayed in Fig.7. In some instances, immobilized laccases can withstand high temperatures, storage, and reusability in a better way than their free counterparts. The control of enzyme immobilization requires a fine control of the process, and many artifacts can occur, that in many instances are ignored by the researcher [125].

289 Nevertheless, immobilization methods have shortcomings along with their
290 advantages (Table 3). Shortly, researchers found that proper immobilization can
291 produce many positive effects on enzyme features. Firstly, immobilization can affect
292 the enzyme stability [126]. Although using an inadequate immobilization protocol
293 (e.g., using a very hydrophobic support) enzyme stability can decrease, using a
294 proper immobilization protocol (that involves support, the groups on the support and
295 their superficial concentration, the immobilization protocol, and the support-enzyme
296 reaction endpoint), the enzyme stability can be greatly increased [127, 128]. This
297 may be achieved mainly if the final support is physically and chemically inert and if
298 an intense multipoint covalent immobilization is achieved, or if all enzyme subunits of
299 a multimeric enzyme are bound to the support [127]. Reported support types for
300 immobilization along with their advantages and disadvantages have been presented
301 in Table 4. If the enzymes are submitted to chemical modification to further improve
302 their features, performing this on immobilized enzymes may be simpler than the
303 modification of free enzymes, making possible enzyme modifications that hardly can
304 be performed in solution by using the benefices of the solid-phase [129].
305 Immobilization may benefit if a proper design of the enzyme surface is performed by
306 site-directed mutagenesis or chemical modification to improve the enzyme
307 immobilization performance, although this synergy has been scarcely exploited in
308 literature [130, 131]. Immobilization can be performed using different strategies.
309 Classically, the immobilization techniques have been classified by the immobilization
310 cause (physical adsorption, covalent bonds, trapping, cross-linking, etc.). However,
311 the current status of this technique suggests that a new classification may be
312 proposed, related to the solid material utilized in the immobilization. There are new
313 proposals to immobilize enzymes using the producing cells, adding to the enzyme

some domain that permits the enzyme to become attached to the cell wall [132]. This strategy is cheap and does not require the extraction of the enzyme, but the loading of the enzyme never becomes very high, and the possibilities of exploring all the possible beneficial effects of immobilization are reduced. A second class of immobilization strategies may be those that do not use a pre-existing solid, but that form an *ex novo* solid. This is composed of copolymers, aggregates, crystals coated with enzymes, nanoflowers, crosslinked enzyme crystals (CLECs) and aggragates (CLEAs), enzymes trapped in solids produced by polymer-formed ex novo (e.g., calcium alginate, lentikats), multifunctional cross-linkable itaconic acid copolymers, sol-gels, and so on [133-137]. The third group of immobilization strategy involve those where a preexisting solid is utilized as a matrix for enzyme immobilization (the immobilization may be via covalent bonds or physical interactions) [138-140]. These supports may be porous or not porous (nanomaterials, membranes) materials, and each of them may have gains and problems, depending on the specific enzyme, application, and reactor [141]. This way, it can be expected that the recycling, operational stability, and resistance to application conditions of laccases may be enhanced when the enzymes are immobilized using an adequate protocol [124]. Depending on the enzyme, the chosen immobilization technique, and preparation conditions, activity recovery varies. Due to higher stability, immobilized laccase can be also more resistant to inhibitors like N_aCl [142, 143]. Despite the common concern of decreased enzyme flexibility, steric hindrance, and diffusion limits, laccase immobilization can sometimes enhance catalytic performance. The efficiency of some laccases in pharmaceutical degradation has been improved by their immobilization on diverse material frameworks [144-146].

Masjoudi et al. [145] reported the removal of carbamazepine and diclofenac by immobilized laccase on a polyvinylidene fluoride nanocomposite containing multi-walled carbon nanotubes. Covalent immobilization of laccase on nanocomposite membrane support was achieved, demonstrating high activity and activity recovery of the immobilized laccase. As a result, the immobilized reusability of laccase was confirmed for five cycles, and its stability was up to 60 °C. The study concluded that immobilized laccase in a mini-membrane reactor demonstrated removal efficiencies of 27% in 48 h and 95% in 4 h for carbamazepine and diclofenac, respectively.

Taheran et al. [144] reported the Immobilization of laccase onto a nanofibrous membrane for the degradation of chlortetracycline (CTC), carbamazepine (CBZ), and diclofenac (DCF) residues in water. The results indicated that the immobilized laccase exhibited superior pH, temperature, and storage stability in comparison to the free laccase. Additionally, it maintained over 17% of its initial activity after 10 cycles of ABTS oxidation, indicating that the enzyme's reusability was improved. The degradation efficiency of three pharmaceutical compounds in batch experiments was 72.7% (DCF), 63.3% (CTC), and 48.6% (CBZ) after 8 hours of reaction when immobilized laccase was employed. Al-sareji et al. [24] reported laccase immobilization on activated carbon derived from pomegranate peels to remove diclofenac, amoxicillin, carbamazepine, and ciprofloxacin from water and wastewater. The subsequent study showed pomegranate peels were successfully used as an adsorbent and enzyme carrier for the removal of emerging contaminants, even from complex sample matrices. The removal of contaminants from effluent was completed in five cycles, while it was extended to six cycles for water.

362 **Table 3:** Reported advantages and disadvantages of laccase immobilization

363 methods.

Laccase immobilization method		Advantages	Disadvantages
Physical	Entrapment	-Simple and rapid -Low cost -No modification of the enzyme	-Low stabilization -Pore diffusion restraint -Enzyme leakage -Difficult to industrial-level deployment
	Adsorption	-Low cost -Straight forward -No modification of the enzyme -Support reusability option	-Low stabilization -Enzyme leakage
	Encapsulation	-Straight forward -Native conformation of the enzyme is kept	-Low stabilization -Highly concentrated enzyme requirement -Mass transfer -Pore size limitations -Enzyme leakage
Chemical	Crosslinking	-Enzyme stabilization -Strong binding -No carrier needed	-Diffusion limitations -Enzyme chemical modification -Crosslinking reagent is required
	Covalent binding	-Prevents leaking -High heat stability -Strong binding - The highest enzyme stabilization	-Complex method -Cost bearing -Chemical enzyme modification

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371 **Table 4:** Reported advantages and disadvantages of support types that have been

372 employed in laccase immobilization [18].

Support type for immobilization	Advantages	Disadvantages
Carbon support	-Easy modification with different functional groups -Electrical conductivity -Good mechanical strength -Considerable adsorption capacity	-Possibility of contamination -High production cost Toxicity
Magnetic support	-Easy surface modification -Good magnetic responsiveness -Easy separation and good reusability	-Susceptible to acidic and oxidative conditions -Agglomeration problem
Inorganic support	-Low cost -Low impact on the environment -High pH and thermal stability -High mechanical strength	-Requirements for adsorption properties of support -Particle size is not easy to change -Enzymes are relatively easy to leak -Modification is required to form strong enzyme support interaction
Synthetic organic support	-Abundant functional groups and easy modification -High enzyme loading -Strong enzyme-support binding -Pore diameter may be chosen -Large specific surface area	-High cost -Non-renewable -Complex synthesis process
Natural organic support	-Low cost, easy to obtain materials -Good biocompatibility and non-toxic -Abundant functional groups and easy modification -Large specific surface area-Pore diameter may be chosen in certain cases	-Possibility of bacterial degradation -Low mechanical rigidity
COFs	-Low density -Large surface area -Regular and orderly pore structure -Structural pre-designability	-Complex synthesis process and cannot be mass-produced -High production cost

	-Good stability and biocompatibility	
MOFs	-High porosity and adjustable pore sizes -Large surface area -Chemical and thermal stability -Multiple functional sites and facile modification	-Negative effect on enzyme activity -High production cost -Microporous channels limit enzyme binding -The metal can act as a catalyst
HNFs	-Low mass transfer limitation -Cooperative effect of enzyme and cation center -Large surface area	-Limited available surface area -Small-scale production -Uneven pore size and porosity

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374 **6. Advances in enzyme immobilization for environmental bioremediation of a**
375 **wide array of pharmaceutically active contaminants**

376 Enzyme immobilization consists of the location of the enzyme in a confined space
377 [121]. This started as a technology intended to simplify the recovery and reutilization
378 of these biological and initially very expensive biocatalysts [122, 123]. Moreover, as
379 with any heterogeneous catalysts, immobilized enzymes can simplify the control of
380 the reactor and increase the reactor types where they can be utilized, as well as
381 simplify the downstream [122, 123]. In the case of bioremediation, the alternative to
382 using an immobilized enzyme in a reactor where the enzyme can be reused (or the
383 reactor can be moved to another contaminated area after being used in one specific
384 place) is the use of a free enzyme. However, the free enzyme will be diluted along
385 the whole medium (a lake, a river, the ocean), making the use of the enzyme less
386 efficient. Its use in soils may be suitable, as very likely the enzyme will become
387 adsorbed in some components of the soil and will perform its function in the desired
388 targeted area, but due to the variability of the components of the different soils, this
389 *in situ* natural immobilization may lead to improvement of enzyme properties in

certain cases, but a worsening of the enzyme features in other cases, depending on the nature of the enzyme-adsorbing materials in the soils (Fig. 8) [147]. The use of immobilized enzymes is a guarantee that these direct interactions with the soil components cannot have a negative effect on the enzyme (Fig. 8), although changes in the composition of the soil composition can affect the concentrations and nature of the salts interacting with the immobilized enzyme, or alter the pH of the medium, etc. and this obviously may affect even to the immobilized enzyme features. However, as stated later in this section, proper immobilization may also help to reduce the impact of these problems. The use of the immobilized enzyme in a reactor (to act in a water medium) can also raise some further advantages. It can permit a certain control of the reaction, perhaps not so strictly as in a standard biocatalytic process, but it may be possible at least to control the reactor temperature. Even, if the reactor is properly designed, and there is easy and cheap access to clean water, it may be possible to mix the contaminated water with this clean water to reduce the concentration of any reagent that can have a negative effect on the enzyme features or have a more neutral pH by reducing the concentration of acid or basic species. Thus, at first glance, enzyme immobilization in bioremediation may have a clear interest, even when the cost cam suggest the contrary [147]. The results of enzyme immobilization require fine control of the whole immobilization process, and many artifacts can occur. In many instances, these artifacts are ignored by the researcher [125] and can lead to wrong conclusions. That way, a deep understanding of the mechanisms of enzyme immobilization and the possible events simultaneously occurring during the immobilization should be considered to take full advantage of the technique and understand the results [125]. Shortly after the launching of the technique, researchers found that proper immobilization may produce many positive effects on

enzyme features. The first objective of enzyme immobilization was to improve enzyme stability, as only enzymes that remain active after use may be reused [126]. Although using an inadequate immobilization protocol (e.g., using a very hydrophobic support) enzyme stability can decrease, using a proper immobilization protocol (that involves proper support, the groups on the support and their superficial concentration, the immobilization protocol, and the support-enzyme reaction endpoint), the enzyme stability can be greatly increased [127, 128]. This may be achieved mainly if the final support is physically and chemically inert and if an intense multipoint covalent immobilization is achieved, or if all enzyme subunits of a multimeric enzyme are bound to the support [127]. A recent review summarizes all possibilities of achieving some enzyme stabilization after its immobilization [148]. Enzyme immobilization may also increase enzyme activity, mainly under drastic conditions (related to higher enzyme stability) (Fig. 9), although in some instances a real positive conformational change that produces an increase in enzyme activity may be found [125]. Immobilization may be coupled to enzyme purification, using protocols defined to achieve this result, with the saving in time, effort, and economic costs that this produces [149]. The fact that the enzyme may be partially distorted and located in a confined space can lead to alterations in enzyme selectivity and specificity. In many instances, using a large enough number of immobilization technologies, a specific immobilized enzyme can greatly improve the properties of the free enzyme for a specific process [150]. Finally, enzyme resistance to inhibitors and distorting agents may be increased, making it possible to use the enzymes under higher substrate concentrations (Fig. 9) [126]. This can have a special interest in bioremediation, where some substrates can produce serious decreases in enzyme activity/stability, even at millimolar concentrations, due to their enzyme inactivation

potential [151] (Fig. 9). The use of enzymes with a more rigid structure via multipoint covalent attachment may prevent the enzyme distortions caused by the substrates, leading this way to the possibility of using the laccase biocatalysts under higher concentrations of substrates and for more time [150] (Fig. 9). It should be noted that several strategies can be simultaneously utilized to improve the final biocatalyst performance, it is not necessary to choose one strategy. In the case of immobilization, the chemical modification of immobilized enzymes may be simpler than the modification of free enzymes, making possible modifications that hardly can be performed in solution by using the benefices of the solid-phase [129, 131, 152]. Enzyme immobilization may benefit if a proper design of the enzyme surface is performed by site-directed mutagenesis or chemical modification to improve the enzyme immobilization performance [129, 131, 152]. However, this synergy has been scarcely exploited in literature [130, 153-156]. Enzyme immobilization can be performed using different strategies. Classically, the immobilization techniques have been classified by the immobilization cause (physical adsorption, covalent bonds, trapping, cross-linking, etc.). However, the current status of this technique suggests that a new classification may be proposed, related to the solid material resulting after the immobilization. The first immobilization class can be formed by the new proposals to immobilize enzymes directly using the producing cells, adding genetically to the enzyme some domains that permit the enzyme to become attached to the cell membrane or wall [132, 157-159]. This strategy is cheap and does not require the extraction of the enzyme, but the loading of the biocatalyst never becomes very high, and the possibilities of exploring all the possible beneficial effects of immobilization are reduced. A second class of immobilization strategies may be those that do not use a pre-existing solid, but that form an ex-novo solid.

This can include copolymers, crosslinked enzyme crystals (CLECs) or aggregates (CLEAs), nanoflowers, sol-gels, crystals coated with enzymes, enzymes trapped in polymer-formed ex novo (e.g., calcium alginate or lentikats beads), etc. [133-135, 137, 160-173]. Finally, the third class of immobilization protocols will be formed by those where a preexisting solid is utilized as a matrix for the enzyme immobilization (the immobilization may be via covalent bonds or physical interactions) [138-140, 174]. These supports may be nonporous (nanomaterials, membranes) or porous materials, and each of them may have advantages and drawbacks, depending on the specific enzyme, reactor, and application [141]. One point usually not considered in immobilization is the possibility of using immobilization techniques where the particle of the biocatalyst can promote the partition of the substrate (Fig. 10) [150]. The increase or the reduction of the concentration of the substrate in the enzyme environment may be interesting, depending on the circumstance. For example, in cases where the contaminant substance to be eliminated is at a very low concentration in the media, far below the enzyme saturation concentration, and that must be maintained that way to prevent damage to the environment, the enzyme performance will be reduced. In this instance, it may be interesting to use a support/modification of the enzyme with polymers where the substrate can become favorably partitioned, and that way to have in the enzyme environment a higher substrate concentration that permits the enzyme to exhibit maximum activity even when the external substrate concentration may be very low (Fig. 10A). In contraposition, if the enzyme may be exposed to occasional high concentrations of some deleterious reagent (the substrate itself or some other component of the medium with negative effects on enzyme stability) (Fig. 9), a partition from this reagent away from the biocatalyst particle may promote positive effects for the

enzyme performance by reducing the concentration of this negative compound in the enzyme environment (Fig. 10B) [27, 150, 175]. Ideally, if the substrate is at low concentration and there are an inactivating/inhibiting compound in the media of very different nature (e.g., one cationic and the other anionic, one hydrophobic and the other hydrophilic), both partition effects could be explored to improve the biocatalyst performance under “real” conditions. Other point to be considered is that some activated supports may behave as “solid” buffers, e.g., supports coated with ionic polymers like polyethyleneimine, and this can help to maintain the enzyme in a favorable pH value when the pH in the medium is fluctuating due to the contamination or by natural factors (Fig. 11) [176]. That way, even when in the laboratory or a controlled bioreactor in a factory, perhaps this may not be relevant; this buffering potential of the polymers can make it recommendable to use them in bioremediation as an immobilization mechanism or to physically modify the immobilized enzyme. Moreover, this modification may have, in some instances, positive effects on enzyme performance (e.g., stabilizing multimeric enzymes, and scavenging some metals that can have negative effects on enzyme features) [176]. However, it should be considered that these hydrophilic polymers may have negative effects on the oxygen concentration in the enzyme environment, and this can negatively affect the performance of the laccase [177, 178]. This way, it can be expected that the recycling, operational stability, and resistance to application conditions of laccases may be enhanced when the enzymes are immobilized using an adequate protocol [124]. Depending on the enzyme, the chosen immobilization technique, and the preparation conditions, final enzyme features vary. Properly immobilized laccases may be able to better withstand high temperatures, and storage behavior and permit reusability better than their free counterparts.

Immobilized laccase can also be more resistant to inhibitors like N_aCl [142, 143]. Despite the common concern of decreased enzyme flexibility, steric hindrance, and diffusion limits, laccase immobilization can sometimes enhance catalytic performance. An array of materials has been reported for laccase immobilization including polyvinylidene fluoride nanocomposite, carbon nanotubes, activated carbon, cetyltrimethylammonium bromide (CTAB)-KOH modified biochar, polyacrylonitrile/polyethersulfone material, glutaraldehyde cross-linked chitosan beads, nanofibrous membrane and so on [24, 124, 144, 179-182]. The immobilization of enzymes on multi-walled carbon nanotubes (MWCNTs) may be improved by the inclusion of carboxylic moieties, according to a recent report [183].

Masjoudi et al. [145] reported the laccase immobilization on polyvinylidene fluoride nanocomposite with multi-walled carbon nanotubes to be employed in the removal of carbamazepine and diclofenac, which exhibited the removal efficiencies of 27% in 48 h for carbamazepine and 95% in 4 h for diclofenac. Al-sareji et al. [24] reported laccase immobilization to remove diclofenac, amoxicillin, carbamazepine, and ciprofloxacin from water and wastewater. Taheran et al. [144] reported covalent immobilization of laccase onto the nanofibrous membrane for degradation of chlortetracycline, carbamazepine, and diclofenac. It was able to exhibit 72.7%, 63.3%, and 48.6% degradation efficiency for chlortetracycline, carbamazepine, and diclofenac respectively, after 8 h of reaction.

7. Deployment of the laccase-biocatalyst system in degrading a wide array of pharmaceutical compounds, and toxicity reduction

The rise in the worldwide population and the concurrent widespread utilization of PhACs (Table 5), including antibiotics, hormones, cardiovascular medications, analgesics, anticonvulsants, anti-inflammatory drugs, and antiepileptic drugs, has

resulted in concerns about water and environmental contamination [184-186]. Most studied toxicological impacts of PhACs have been reported in Table 6. Consequently, these pollutants often evade treatment facilities and permeate further into natural water sources such as groundwater, rivers, and water bodies, ultimately contaminating drinking water supplies [187, 188]. As a result, there has been a rise in the study and implementation of biological wastewater treatment techniques with the purpose of PhACs removal. In recent years, laccase has been deployed in the degradation of diverse PhACs [37, 189-194]. A few examples of PhAC degradation by applying laccase in free and immobilized form have been explained in detail in subsequent sections. Recently reported laccase-assisted PhACs degradation is summarized in Table 6. The effective function of laccase for lowering toxicity and the degradation of pharmaceuticals is evident in observations from recent studies [195, 196]. Laccase-based pharmaceutical degradation has been recognized as an economically viable method for the complete biotransformation of antibiotics from aquatic media [196]. Furthermore, the agar-diffusion method demonstrated that biodegraded products were non-toxic and promoted the growth of *Staphylococcus aureus* and *E. coli* [195]. Feng et al. [197] reported the transformation of atenolol (ATL) by the native laccase from *Trametes versicolor* in an aqueous solution. In a subsequent study, the toxicity of ATL and TEMPO mixtures was significantly reduced through laccase treatment. The aforementioned findings suggest that the reduction of pharmaceuticals by laccase into less toxic transformed compounds is a sustainable and eco-friendly method.

Table 5 Compilation of identified PhACs in wastewater systems on a worldwide scale throughout recent years.

ɔuntry wise eo-location	**Pharmaceutical compound**	**Concentration**	**Wastewaters/sources**	**Reference**

Iran	Carbamazepine	16.87 mg L^{-1}	Real pharmaceutical wastewater	[90]
	Ibuprofen	17.26 mg L^{-1}		
	Azithromycin	10.62 mg L^{-1}		
	Nalidixic acid	15.20 mg L^{-1}		
India	Ketoprofen	3–41 µg L^{-1}	Domestic and hospital wastewater	[90]
	Aspirin	125–184 µg L^{-1}		
	Diclofenac	12–68 µg L^{-1}		
	Naproxen	11–217 µg L^{-1}		
	Ibuprofen	5–22 µg L^{-1}		
	Enrofloxacin	780–900 µg L^{-1}	Households and hospitals	
	Ciprofloxacin	28000–31000 µg L^{-1}		
	Cetirizine	1300–1400 µg L^{-1}		
	Enoxacin	150–300 µg L^{-1}		
Korea	Sulfamethoxazole	194 ng L^{-1}	Urban, domestic sewage, and industrial wastewater	[90]
	Trimethoprim	21 ng L^{-1}		
	Erythromycin	44 ng L^{-1}		
audi Arabia	Trimethoprim	0.05-4.8 µg L^{-1}	WWTP-1 influent	[198]

	Ciprofloxacin	7.1-711.3 μg L^{-1}		
	Diclofenac	0.4-45.3 μg L^{-1}		
	Acetaminophen	46.4-943 μg L^{-1}		
	Ofloxacin	0.9-88.2 μg L^{-1}		
	Metformin	3.1-587.9 μg L^{-1}		
China	Roxithromycin	34.14-353.33 ng L^{-1}	WWTPs	[199]
	Ofloxacin	47.87-491.53 ng L^{-1}		
	Sulfamethoxazole	121.98-275.04 ng L^{-1}		
	Tetracycline	14.17- 104.23 ng L^{-1}		
	Norfloxacin	84.89-458.49 ng L^{-1}		
	Sulfadiazine	87.67-145.70 ng L^{-1}		
	Oxytetracycline	36.22-256.70 ng L^{-1}		
Nigeria	Norfloxacin	561 μg L^{-1}	Hospital wastewater	[90]
	Ofloxacin	198 μg L^{-1}		
	Ciprofloxacin	228 μg L^{-1}		
Spain	Ofloxacin	592.9–14377.8 ng L^{-1}	Hospital and urban wastewater	[90]
	Ciprofloxacin	639.1–8372.9 ng L^{-1}		
	Cefazolin	83.4–94.7 ng L^{-1}		

	Acetaminophen	18–74 μg L^{-1}		
Canada	Ibuprofen	49000 ng L^{-1}	Municipal, hospital wastewater, urban and industrial wastewater	[90]
	Enrofloxacin	12 ng L^{-1}		
	Ciprofloxacin	600 ng L^{-1}		
	Tetracycline	53 ng L^{-1}		
	Acetaminophen	104–105 ng L^{-1}		
Colombia	Ketoprofen	0.12–0.16 μg L^{-1}	Urban wastewater	[90]
	Gemfibrozil	2.7–3.2 μg L^{-1}		
	Carbamazepine	0.17–0.19 μg L^{-1}		
	Ibuprofen	6.4–19.0 μg L^{-1}		
France	Cyclophosphamide	0.5–0.8 μg L^{-1}	Hospital wastewater	[90]
	Sulfamethoxazole	12.3–33.5 μg L^{-1}		
	Atenolol	1.6–6.5 μg L^{-1}		
South Africa	Aspirin	118 ± 0.82 μg L^{-1}	Domestic, hospital, commercial, and industrial wastewater	[90]
	Diclofenac	22.3 ± 0.63 μg L^{-1}		

564

565

566 **Table 6** Deployment of laccase in degradation of a broad array of PhACs.

Laccase source	Deployed enzyme form	Application in PhACs degradation	Reaction mechanism & parameters	Removal efficiency	Reference
Trametes versicolor	Magnetically modified biochar immobilized laccase	Norfloxacin, enrofloxacin and moxifloxacin	pH 4 and 40 °C after 48 h reaction	93.7 %, 65.4 % and 77.0 %	[200]
Trametes versicolor	Immobilization using electrospun materials	Tetracycline	pH 5, 25 °C	100% and 94% for covalently bonded and encapsulated laccase	[201]
Aspergillus species	Immobilized on zeolitic imidazolate frameworks	Carbamazepine	pH 7.0, 20–70 °C	~92%	[146]
Aspergillus species	Immobilized on a zeolitic imidazolate framework	Diclofenac and norfloxacin	pH 6.4	93.9 and 95.1%	[202]
Trametes hirsuta	Immobilized on polyvinylidene fluoride membrane modified with multi-walled carbon nanotubes	Carbamazepine and diclofenac	pH 5, 25 °C	27% in 48 h and 95% in 4 h were obtained for carbamazepine and diclofenac	[145]
Trametes versicolor	Immobilized on date stones	Ketoprofen and aspirin	pH range 2–7.5, 25 °C	Complete removal within 4 h of treatment	[203]
Pleurotus ostreatus and *Lentinus sajor-caju*	Free	Sulfamethoxazole	pH 5.0, 25 °C	Approximately 100 % of SMX degradation was attained in 30 min	[196]

Pleurotus florida	Free	Ciprofloxacin and norfloxacin	pH 4.5, 30 °C	Ciprofloxacin (86.12–75.94%) and norfloxacin (83.27–65.94%) was achieved	[195]
Trametes versicolor	Free	Atenolol	pH of 7.0, 25–50 °C	Transformation efficiencies are 77%, 100%, and 100% with the addition of 500 µM TEMPO after 4, 12, and 24 h enzyme treatment, respectively.	[197]
Pleurotus ostreatus	Free	Clomipramine, mianserin, paroxetine, sertraline, and mycophenolic acid	pH 6.5, 26 °C	Sertraline (5, D(4h) = 91.2 ± 3.3%, D(96h) = 92.8 ± 4.5%), paroxetine (3, D(4h) = 86.1 ± 6.2%, D(96h) = 93.7 ± 4.2%), clomipramine (2, D(4h) = 89.6 ± 4.2%, D(96h) = 98.4 ± 0.3%) and mianserin (4, D(4h) = 63 ± 10%, D(96h) = 94.01 ± 0.64%). Mycophenolic acid was removed	[204]

				after 4 h completely	
Trametes versicolor	Free	Doxorubicin	pH 7, 30 °C	Reduction of 41.4% doxorubicin toxicity	[192]
Trametes versicolor	Graphene Facilitated laccase	Labetalol	pH 7.0	Complete removal was 90 min while the concentration of ABTS was 5 or 10 μM	[205]

567

568 **7.1 Degradation of anticancer drugs**

569 The detection of anticancer drugs in hospital wastewater and also surface water
570 samples has raised worldwide concern. A number of anticancer drugs have been
571 identified on a global scale in the environment, including doxorubicin, etoposide,
572 fluorouracil, cyclophosphamide, ifosfamide, tamoxifen, vinblastine and vincristine
573 [192, 206-209]. Kelbert et al. [192] reported the degradation of doxorubicin, an
574 anticancer drug, by direct application of laccase. The doxorubicin underwent with
575 most noticeable enzymatic degradation at pH 7 and 30 °C, which closely resembles
576 the properties of effluent from wastewater treatment plants (that obviously, cannot be
577 controlled) [192]. Maximal velocity (Vmax) of 702.8 μgDOX h^{-1} L^{-1} and Michaelis-
578 Menten constant (K_M) of 4.05 μM were the Michaelis–Menten kinetic parameters
579 acquired for this reaction. The K_M value indicated a good affinity for the substrate.
580 Cell (L-929) viability is reduced by 27% in the presence of doxorubicin (1000 μg L^{-1}).
581 Laccase degraded doxorubicin into non-toxic compounds, as evidenced by the
582 41.4% reduction in toxicity of doxorubicin at the maximum concentration tested
583 (1000 μg L^{-1}). Pereira et al. [23] documented the enzyme-mediated degradation of
584 etoposide employing various laccases at different pH values. Subsequent research

revealed that etoposide was completely degraded in 60 minutes by a laccase, using activity of 1100 UL^{-1}, and that using a laccase activity of 55 U L^{-1}, 86% of the etoposide after 360 minutes could be removed. Similar to conditions found in wastewater treatment plants (pH 6 and 7), etoposide was degraded by laccase at all pH-studied pH vales.

Jinga et al. [210] reported an effective method employing Laccase-TEMPO for removing doxorubicin from wastewater. A subsequent study revealed that various ratios of doxorubicin, laccase, and TEMPO were used to get results (shown as % of elimination) at pH 5 and 7, 2, 4, 6, and 24 hours [210]. Experiments showed that larger catalyst concentrations and longer reaction times resulted in greater removal efficiencies (up to 100%). This demonstrated that the anticancer drug doxorubicin may be effectively removed from wastewater using the laccase-TEMPO biocatalytic system.

7.2 Degradation of analgesics/NSAIDs

Analgesics, which include both opioid and non-opioid formulations, function as pharmaceutical agents that alleviate pain and that may be found in wastewater [211, 212]. Laccases have undergone evaluation against nonsteroidal anti-inflammatory drugs, including aspirin and ketoprofen [14, 203]. The excessive use of aspirin (acetylsalicylic acid), an anti-inflammatory drug commonly prescribed for the treatment of pain and fever, has become a significant environmental pollution concern. This has detrimental consequences for aquatic organisms, which include reproductive and fetal development [14]. Multiple research studies have shown the effectiveness of free laccases in the degradation of NSAIDs [189, 213-215].

Al-Sareji et al. [203] reported the utilization of immobilized laccase on date stones to eliminate ketoprofen and aspirin. In the following study, aspirin, and ketoprofen biodegradation in aqueous solutions were investigated via batch experiments. The laccase retained 54% of its original activity after six cycles, as determined by oxidation assays using ABTS [203]. Following that, an immobilized laccase system was implemented to catalyze the degradation of 25 mg L^{-1} of ketoprofen and aspirin, which nearly entirely disappeared within 4 h of treatment. Coman et al. [213] reported the degradation of sodium diclofenac by laccase from *Sclerotinia sclerotiorum*. Subsequent research showed that much of diclofenac degradation (>96%) occurred via radical-generated oligomers and their rapid precipitation after a 30-hour treatment, thereby establishing an unprecedented green formula appropriate not only for degradation but also for the straightforward elimination of degradation products.

Apriceno et al. [191] reported the degradation of NSAIDs (ketoprofen (KP), naproxen (NAP), and diclofenac (DCF)) exploiting direct immobilization on chitosan beads of a periodate-oxided laccase from *Trametes versicolor*. As it turned out, the ideal experimental conditions for DCF degradation at 90% after 3 hours were pH 3 and a 1:1 M ratio for ABTS: drug. The combination of DCF, naproxen, and KP was used to assess the continued effectiveness of laccase in removing DCF and potentially focusing on the other drugs. With only 0.02 U of laccase activity, DCF was degraded completely in a period of three hours. After seven days of degradation, the hydroxylated compounds that emerged as the transformed products of DCF were identified. In contrast, it was found that the quantity of NAP-degraded products was drastically reduced.

Ratanapongleka et al. [216] reported degradation of acetaminophen-contaminated aqueous solution to be optimized by the use of immobilized laccase conditions. Barium alginate was used to immobilize the laccase from *Lentinus polychrous*. Sodium alginate 5% (w/v), barium chloride 5% (w/v), and a 60-minute gelation period were the optimum immobilization conditions. As the concentration of the enzyme escalated, there was a corresponding increase in both the degradation rate and the percentage of removal. In 240 minutes, immobilized laccase at 0.57 U/g-alginate achieved 94% removal. The immobilized enzyme demonstrated excellent acetaminophen removal and high activity at pH 7 and 35 °C. For acetaminophen degradation, the activation energies of free and immobilized laccase were 8.08 and 17.70 kJ/mol, respectively. Furthermore, the immobilization of laccase increased its stability to changes in pH and temperature. Furthermore, immobilized laccase could be possibly reused for up to five cycles.

7.3. Degradation of antibiotics

Antibiotics are employed in livestock farming and human and veterinary medicine, thus constituting one of the most widely utilized classes of medications worldwide [217-220]. Antibiotics that do not undergo decomposition are persistent and get into the environment. Furthermore, the primary challenge with antibiotics is the emergence and spread of resistant bacteria, which has been rendered easier considering the present situation [221]. Antibiotics are not effectively removed either by conventional water treatment processes or advanced treatment methods; although advanced remediation methods are more efficient, they come with drawbacks, including high costs and resulting in secondary pollution [222, 223]. As "green and sustainable biocatalysts" for antibiotic degradation, laccases have proven great potential for the degradation of diverse classes of antibiotics [224]. The primary

emphasis has been on developing and deploying immobilized laccase to address the existence of antibiotics in environmental matrices [224-227].

Harguindeguy et al. [228] reported the degradation of tetracycline (TC) using immobilized laccase from *Trametes versicolor* in a fluidized bed reactor. Immobilized laccases exhibited better thermal and pH stabilities than those of free laccases. Subsequently, the highest degradation rate obtained was 72 ± 1%, with a circulation flow rate of 80 mL min^{-1} and air bubbling at 15 mL min^{-1}. A study of the immobilized enzyme stability under reaction conditions revealed that 45% of the TC was degraded after 5 cycles of 24 hours each. Microtox assays were used to examine the toxicity of the TC solution before as well as after treatment. Subsequent microtox tests revealed that enzymatic degradation decreases the acute toxicity of water.

Sá et al. [196] reported the biotransformation of sulfamethoxazole (SMX) catalyzed by different laccases. *Pleurotus ostreatus* and *Lentinus sajorcaju* were used to produce laccases utilizing agricultural and food residues as substrates, notably vine pruning and brewer spent grains. The produced fungal laccases were subsequently evaluated for their ability to degrade SMX in aqueous environments, with and without the presence of natural redox mediators. Laccase derived from *Lentinus sajor-caju* exhibited the highest rate of SMX transformation; that is, it degraded approximately 100% of SMX (SMX (200 mg L^{-1}) and the mediators SYR and PCA (100 mmol L^{-1})) in 30 minutes.

Ouyang et al. [229] pointed out the effective removal of sulfonamides and tetracycline residues by the laccase-mediator system using a novel laccase from *Lysinibacillus fusiformis*. A novel laccase from *L. fusiformis* (Lyfu-Lac) revealed promising removal effectiveness onto sulfonamides and tetracycline residues in the

presence of syringic acid (SA) and ABTS. After 12 hours, the Lyfu-Lac-SA system removed over 85% of sulfamethazine (SMZ), sulfamethoxazole (SMX), and sulfadiazine (SDZ) at 40 °C. In contrast, oxytetracycline (OTC) and tetracycline (TC) were reduced by 16.9% and 0%, respectively, after 6 hours using the Lyfu-Lac-ABTS system.

Wang et al. [230] reported the elimination of tetracyclines in seawater by a laccase-mediator system. Laccase was utilized from *Aspergillus* sp. To degrade tetracyclines (TCs) in coastal seawater at environmentally significant concentrations (ngL^{-1}-μgL^{-1}), mediators that incorporated distinct oxidation mechanisms were combined. The enzymatic structure of laccase was altered by the elevated salinity and alkalinity of seawater, leading to a decreased affinity for the substrate (Km of 0.0556 $mmolL^{-1}$) of laccase in seawater compared to buffer (Km of 0.0181 $mmolL^{-1}$). Despite the decline in stability and activity of laccase when exposed to seawater, it was possible to completely degrade TCs in seawater within two hours using laccase at a concentration of 200 UL^{-1} coupled with a laccase/syringaldehyde (SA) ratio of 1 U:1 µmol, even at initial concentrations of less than 2 μgL^{-1}. Sarnthima et al. [231] molecular insight of sulfamethoxazole degradation using laccase from *Streptomyces* sp. CS29. The activity of laccase was shown to be enhanced by 10 and 20 mM Ca^{2+}, 20 mM Zn^{2+}, and 10 mM K^{+}. Based on the results of the following investigation, the best pH for breaking down sulfamethoxazole was 3.0, and the degradation rate was 97.90%.

7.4 Degradation of antiepileptic agents

Carbamazepine (CBZ) is a PhAC with antiepilectic effects [232-234]. This is one of the most resistant PhAC compounds routinely identified in wastewater effluent-contaminated environments. Moreover, CBZ exhibited resistance to removal via

flocculation, coagulation, and chlorination processes [235]. That way, biocatalytic degradation utilizing enzymes such as laccases presents a potentially effective strategy for the complete removal of CBZ from water matrices [214, 235-237]. The ineffectiveness of recent laccase-based investigations to degrade CBZ was attributed to the electron-withdrawing group amide present in the structure of CBZ [235]. This functional group rendered CBZ strongly electron-deficient and reduced its laccase oxidation potential [235]. Application of redox mediators, with a more suitable potential, including ABTS and HBT, can augment the oxidation capacity of laccase to cope with this concern [235].

Simón-Herrero et al. [237] reported the removal of CBZ by immobilized laccase on polyimide aerogels. Subsequent findings indicated that the activity of immobilized laccase on polyimide aerogels was significantly improved in acidic or basic pH conditions when compared to that of the free enzyme. Additionally, the activity of the immobilized enzyme was greater than that of the free enzyme form across all of the temperature ranges. Owing to the immobilization of this support material, the storage stability was also improved. According to reusability experiments, the immobilized laccase retained 22% of its original activity after 7 cycles when it was employed to oxidize ABTS. 76% and 74%, respectively, of CBZ degradation were achieved by immobilizing laccase on polyimide aerogels in spiked water and secondary effluent. In addition, the CBZ removal efficiency remained elevated even after 7 cycles (65% for secondary effluent and 50% for spiked water, respectively).

Naghdi et al. [238] reported the removal of CBZ from immobilized laccase on oxygen-functionalized nanobiochars. The impact of applying HCl, H_2SO_4, HNO_3, and their mixtures to oxidize nanobiochar, a carbonaceous material generated through

biomass pyrolysis, on the immobilization of laccase was investigated in the subsequent study. The storage, pH, and thermal stability of immobilized laccase on functionalized nanobiochar were found to be superior to those of free laccase, indicating that this material had the capacity to be utilized continuously. Reusability investigations on ABTS oxidation revealed that 70% of the initial activity of the immobilized laccase was retained after three cycles. As a result, the immobilized laccase utilized for CBZ degradation achieved 83% and 86% removal, respectively, in spiked water and secondary effluent.

Dlamini et al. [146] reported aspergillus-based laccase immobilization for the biocatalytic degradation of carbamazepine. Zeolitic imidazolate frameworks (ZIF) were utilized as efficient porous substrates for attaching laccase enzymes. Additionally, the synergistic adsorption and biocatalytic degradation of CBZ in aqueous solutions were investigated. The results of CBZ degradation revealed that immobilization of the laccase enhanced its resistance and stability at different pH levels when compared to the enzyme in its free form. In contrast to the free form, the immobilized laccase evidenced comparatively elevated activities throughout the temperature range under investigation. Vmax, which was determined to be 0.873 and 0.692 mg L^{-1} h^{-1} for the free and immobilized laccase, respectively, exhibited a negligible decrease after immobilization, as indicated by kinetic investigations. The enhanced solubility of the immobilized laccase in organic solvents enables the composite to be utilized in real wastewater samples. The efficacy of the laccase-ZIF composite in decontaminating CBZ was demonstrated to be approximately 92%. Moreover, the immobilized laccase evidenced noteworthy stability in storage (approximately 70% residual activity) for a duration of 15 days before encountering any substantial decline in activity.

7.5 Beta blocker degradation

Beta-blockers are classified as adrenergic antagonists, primarily employed to treat hypertension, congestive heart failure, and abnormal cardiac arrhythmias [239]. The concentration of beta blockers in wastewater, surface waters, and groundwater has been reported to vary significantly from ngL^{-1} to μgL^{-1}, indicating their ubiquitous presence [240-242]. According to reports, beta-blockers induce chronic toxicity in aquatic organisms, which implies that their continued introduction into the environment could be detrimental to both humans and the environment [240]. Beta-blockers may remain in surface waters and groundwater as a result of their relatively inefficient rate of elimination from the natural environment. In spite of the shortcomings of physicochemical removal technologies, which necessitate substantial energy and reagent inputs, and even lower safety levels, hazardous byproducts may be produced during the oxidation process [243]. Therefore, enzymatic remediation is a more viable alternative for the removal of beta-blockers from water matrices [55, 197, 205].

Dong et al. [244] reported laccase-graphene composite potential in the removal of labetalol. In further investigation, pristine few-layer graphene (FLG) was employed to interact with laccase to synthesize a laccase-graphene composite designed to remove labetalol. The synthesized laccase-FLG composite had an enzyme loading dosage of 221.1 mg g^{-1}. In summary, the laccase-graphene composite has resulted in the potential to be repurposed for the removal of labetalol more than ten times.

Feng et al. [197] reported the transformation of atenolol (ATL) by the native laccase from Trametes versicolor in an aqueous solution. The removal efficacy of

ATL via laccase-catalyzed reaction was analyzed in the presence of a variety of laccase mediators. The results indicated that only the mediator TEMPO was capable of significantly facilitating ATL transformation. The ATL transformation was highly pH-dependent, with an optimal pH of 7.0, and it remained nearly constant within a temperature range of 25–50 °C. In a subsequent study, the toxicity of ATL and TEMPO mixtures was significantly reduced through enzymatic treatment.

Dong et al. [205] reported labetalol removal employing the laccase-ABTS System. The study revealed that labetalol can be effectively transformed through a laccase-catalyzed reaction with ABTS as a mediator. However, in the absence of ABTS, no significant removal of labetalol could be achieved. In conclusion, the concentration of ABTS was either 5 or 10 μM, and the time required to achieve complete labetalol removal was 90 minutes. The transformation was substantially accelerated when 25 μM ABTS was present, necessitating only approximately 60 minutes to achieve the complete removal of 5 μM labetalol.

8. Laccase mediator system to enhance the catalytic process for a diverse range of pharmaceuticals

Laccase has the ability to catalyze the oxidation of numerous organic compounds. Despite the employment of molecular oxygen, it is considered a green environmentally friendly, cost-efficient, and safe enzyme catalyst [245]. Nevertheless, there are still some issues that need to be resolved when laccase is explicitly employed in environmental remediation. Initially, a significant number of non-phenolic substrates were unable to directly bind to laccase. Secondly, the redox potential of the majority of laccases (0.5-0.8 V) seemed insufficient to oxidize high-potential phenolic compounds and other complex non-phenolic compounds [246]. Schematic illustration of the laccase-mediator model, and its

deployment in Sulfamethoxazole degradation is portrayed in Fig. 12. Currently, synthetic mediators that are frequently used include ABTS, 1-hydroxy benzotriazole (HBT), 2,2,6,6-tetramethylpiperidine oxide (TEMPO), *n*-hydroxy-*n*-acetylaniline (NHA), and phenothiazine (PT) [245]. Further, researchers have discovered that a diverse array of small-molecule natural compounds, including syringaldehyde (Sa), acetosyringone (As), and vanillin (Va), can function as laccase mediators [245, 247]. Since non-phenolic substances, such as pharmaceuticals, often have a greater redox potential than the majority of laccases produced by fungi, they are not necessarily appropriate for laccase oxidation [246]. Also, the alteration of the substrate may be significantly influenced by the presence of certain functional groups in its structure. Substrates are more susceptible to battery by laccase since they include functional groups, often known as electron donating groups, such as hydroxyl and amines [246]. It is possible for laccase to oxidize mediators into free radicals [247]. Owing to their lack of specificity, these radicals have the ability to oxidize additional contaminants, expanding the range of chemicals that might be broken down by laccase. In the process of oxidizing a pollutant, mediators sometimes called "electron shuttles", may be reduced back to their original composition after being oxidized to radicals by laccase [247]. Recent studies have shown that certain small molecule mediators may mediate the oxidation reaction between laccase and substrate (pharmaceuticals) [226, 247-250]. The scope of laccase may be further expanded by this modification.

Parra Guardado et al. [246] reported an influence of redox mediators on pharmaceutical degradation by laccase from *Pycnoporus sanguineus* CS43 that was investigated against the commercial laccases *Trametes versicolor* and *Myceliophtora thermophile*. The subsequent investigation revealed micropollutants were resistant to

degradation in the absence of redox mediators, except the antibiotic amoxicillin, which was transformed by all exploited laccases. The results indicated that the degradation of a complex combination of pharmaceuticals was influenced by both the compound and the redox mediator. The antibiotics amoxicillin (80%), sulfamethoxazole (100%), and ciprofloxacin (40%) exhibited the highest degradation yields with syringaldehyde serving as the most effective redox mediator within a 3 hours treatment period. Compared to *P. sanguineus* CS43 laccase, commercial laccases demonstrated superior catalytic performance, particularly in the presence of redox mediators. The potential of these systems to remove complex contaminant matrices is demonstrated by the effective transformation of pharmaceuticals through the combined action of various laccases and redox mediators.

Naghdi et al. [235] reported biotransformation of carbamazepine by the laccase-mediator system, which affected the removal of carbamazepine with the laccase-ABTS system by temperature and pH. In a subsequent study, it was reported that the highest degradation efficiency of carbamazepine with laccase-ABTS was up to 95%. Conversely, laccase from *Trametes versicolor* is unable to achieve a degradation efficiency of more than 32% in the absence of ABTS. Ghose et al. [195] reported ciprofloxacin and norfloxacin remediation from wastewater through laccase using ABTS. In a subsequent study, it was demonstrated that the degradation of ciprofloxacin (86.12–75.94%) and norfloxacin (83.27–65.94%) was effective within 3 hours at a temperature of 30 °C, pH 4.5, and ABTS (0.05 mM).

9. Computational-aided pre-screening-based approaches for the degradation of unexplored pharmaceuticals

Extensive research has been conducted on the vital role of laccases in the degradation of xenobiotics, consisting of phenols, anilines, and PhACs [43, 179, 219, 251, 252]. Despite its broad substrate specificity, it enables numerous screening opportunities for the sustainable removal of PhAC in a sustainable way [14, 253, 254]. Despite advancements in numerous approaches to pollutant removal, which include enzyme-conjugated nanocomposites and immobilized enzymes, a significant concern persists concerning their complete degradation and eventual fate in the environment, and left over the toxicity after experimental operation [63, 255, 256]. These concerns pertain to the fate of completely transformed metabolites, the active site amino acid responsible for catalysis, and molecular interaction among amino acids and pollutants, which remain unsolved in conventional remediation methods [61, 63, 107, 257]. Computational degradability prediction of concerned PhACs with a possible enzyme is a robust approach that could deal with inadequacies in a joint effort by validating such outcomes through a conventional real-time degradation assay under controlled settings [60, 63, 258, 259]. Such aforementioned degradability predictions include docking, MD-Simulation, and degradation pathways prediction that have been employed to unravel the degradation process at the atomic level [60, 108, 260]. Docking methods are often used to identify the best conformational state of the docked complex (enzyme-pollutant), as well as the binding contact between the pollutant and the enzyme's active site [57, 61, 107, 108, 261-263]. Enzyme-pollutant docking analyses facilitate the visualization and estimation of the most frequent amino acid residues involved in pollutant binding, as well as the straightforward determination of chemical bonds between the corresponding enzyme-pollutants [107, 261]. However, docking alone is insufficient for exhibiting the actual catalytic activity of enzyme-contaminants in real-time [61,

62]. An MD simulation may be undertaken to observe the real-time conformational behavior of enzyme and pollutant bound complex using an appropriate time-scale simulation run under a specific build model system (i.e. NPT or NVT) [61]. MD simulation offers valuable information on how enzymes or proteins interact with pollutants (ligands), focusing on the chemical bond formation and the role of active site residues in the degradation process, including the post-simulation energy analyses, and system equilibrium state [61, 260]. Such functionality could be implemented to predict the degradability and catalytic potential of an enzyme toward targeted PhAC. Nonetheless, protein engineering techniques could be used to improve the enzyme's capability to better binding and catalytic attributes [264-266]. The proposed computational framework for the binding and chemical functionalities of PhACs in the context of the degradation mechanism, which is based on laccase, can be seen in Fig. 13. A clear binding amino-acid residues and Gibbs free energy (ΔG) assessment can be observed through above explanatory illustration. In the context of the above functionalities, physico-chemical properties of laccase from varying origins may or may not be comparable, which ultimately influences the binding and degradation of target contaminants under specific environmental conditions. Consequently, it is of the utmost importance to comprehend the various parameters of physico-chemical properties. Therefore, diverse physicochemical properties of laccases have been summarized in Table 7.

Nawaz et al. [267] reported bioremediation potential of laccase from *Bacillus ligniniphilus* L1 in set of 18 antibiotic degradation (penicillin, levofloxacin, cephalosporin, tobramycin, linezolid, clindamycin, metronidazole, chloramphenicol, nitroimidazole, fosfomycin, tetracyclines, rifamycin, vancomycin, daptomycin, sulfonamide, trimethoprim, polymyxin, and colistin). AutoDock predicted that the

908 binding energies of the 18 antibiotics with laccase range from −3.7 to −8.1 kcal mol^{-1}.
909 A total of six antibiotics, including vancomycin, levofloxacin, tetracycline, rifamycin,
910 linezolid, and tobramycin, were chosen for MD simulation and experimental
911 validation with laccase. Subsequent research revealed that laccase-vancomycin,
912 levofloxacin, tetracycline, rifamycin, linezolid, and tobramycin were the top-ranked
913 complexes of laccase that were validated through 250 ns MD-simulation by
914 employing the AMBER tool. The research concluded that the computational
915 technique is useful for studying antibiotic degradation by enzymes, which may help
916 with environmental contamination remediation.

917 Mora-Gamboa et al. [57] reported *In silico* prediction of 5 antibiotics
918 (Levofloxacin, Sulfisoxazole, Cefuroxime, Cephradine, and Tetracycline)
919 biodegradation employing laccase from *Ganoderma lucidum* GILCC 1 origin. A
920 subsequent study concluded a high affinity for Levofloxacin (−8.2 kcal mol^{-1}),
921 Sulfisoxazole (−7.8 kcal mol^{-1}), Cefuroxime (−7.5 kcal mol^{-1}), Cephradine (−7. 5 kcal
922 mol^{-1}), and Tetracycline (−7.5 kcal mol^{-1}), attributed to pocket topology and
923 interactions such as hydrogen bonds and van der Waals forces with laccase.
924

925 **Table 7** Comparison of the physicochemical features of laccases from various sources.

Laccase origin species	PDB	Amino-acids	Molecular formula	Molecular weight (Da)	Negatively charged residues (Asp + Glu)	Positively charged residues (Arg + Lys)	Theoretical pI	The instability index (II)	Aliphatic index	Grand average of hydropathicity (GRAVY)
Trametes versicolor	1KYA	499	$C_{2399}H_{3600}N_{638}O_{729}S_9$	53331.35	45	20	4.69	26.88	81.34	-0.028
Streptomyces carpinensis	8AIP	293	$C_{1407}H_{2148}N_{412}O_{427}S_{16}$	32180.00	37	37	5.95	41.39	63.45	-0.495
Streptomyces viridosporus	3TBB	313	$C_{1498}H_{2280}N_{444}O_{456}S_{11}$	34157.94	41	31	6.16	34.54	60.73	-0.657
Thermus thermophilus HB27	6Q29	439	$C_{2210}H_{3489}N_{613}O_{602}S_{14}$	48727.60	48	47	7.09	41.29	96.83	-0.146
Coriolopsis trogii	2HRH	496	$C_{2382}H_{3604}N_{636}O_{729}S_9$	53103.18	48	23	4.83	36.55	82.80	-0.082
Cerrena caperata	4JHU	496	$C_{2387}H_{3609}N_{643}O_{729}S_{11}$	53330.44	47	23	4.87	35.38	80.83	-0.127
Streptomyces griseoflavus	7PEN	322	$C_{1511}H_{2319}N_{451}O_{462}S_{13}$	34611.56	37	27	6.06	33.75	66.96	-0.433
Melanocarpus albomyces	3FU7	559	$C_{2764}H_{4153}N_{759}O_{831}S_{15}$	61791.87	62	34	4.91	32.71	78.59	-0.325
Trametes maxima	2H5U	499	$C_{2384}H_{3571}N_{653}O_{734}S_7$	53347.93	38	21	5.26	30.41	73.51	-0.207

Coriolopsis gallica	4A2E	496	$C_{2371}H_{3579}N_{631}O_{722}S_9$	52763.83	45	21	4.84	31.42	83.25	-0.023

926 927 Physicochemical properties have been predicted using the ProtParam – Expasy tool by utilizing amino acid sequences from corresponding PDB IDs.

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10. Concluding remarks and future outlook

A wide range of PhACs including, anticancer drugs, analgesics/NSAIDs, antibiotics, antiepileptic agents, and beta-blockers as micropollutants often exist in water matrices as a result of consumption and excretion through different routes. PhACs have been detected in water systems in various regions of the globe at concentrations as low as ng-µg. The introduction of PhACs into water matrices is a significant source of public and environmental health concerns for the global population that is exposed to such contamination. The detrimental effects of these PhACs on the ecosystem have been extensively investigated in aquatic organisms, such as phytoplankton, fish, daphnia, and crustaceans. PhACs are not only detrimental to aquatic organisms but they are also associated with antibiotic resistance, which is a growing concern for humans. Nevertheless, there is a lack of a reliable regulatory framework to reduce the hazards associated with pharmaceutical-based water contamination as a means to achieve clean water. However, there are various physical-chemical water treatment and contaminants remediation technologies that are currently in existence. These technologies have limitations in certain aspects, such as cost, and they are not feasible for scaling up. Enzymatic remediation techniques are more eco-friendly and reliable than those mentioned above because they do not produce toxic substances during the experimental operation and implementation. To address these limitations, laccases, a multicopper O2-dependent biocatalyst, have been implemented in the degradation of a diverse array of contaminants, including PhACs, which are frequently detected in water matrices. Laccases that promote the oxidation of various contaminants in the presence of molecular oxygen may need natural and synthetic mediators to increase catalytic reactions for the degradation of PhACs. Laccase-based biocatalyst

systems, both free and immobilized, have been used in several studies on the degradation of PhACs under controlled conditions. The catalytic efficiency and broad acceptance of laccase for the breakdown of PhACs have garnered considerable interest among researchers. Mainly bacterial and fungal laccases have been utilized in experimental degradation as free-form, along with a wide range of mediators. Although free-form enzymes are often challenging to extract from liquid samples, they can only be utilized once for water treatment applications and the location can be hard to fix. This results in an increase in the overall cost of the process since a larger amount of enzymes need to be produced. In addition, highly polluted wastewater, under thermal and pH natural changes, often reduces the stability and activity of free enzymes. Laccase immobilization may solve some of these problems. Adsorption, encapsulation, and covalent bonding are only a few of the strategies looked into for immobilizing laccases on nanomaterials, membranes, and fibers. However, resources that are cost-bearing and non-hazardous to the environment are still needed for the effective elimination of pollutants. In recent years, a smart computational degradability prediction method has been adopted to better understand the degradation mechanism of antibiotics and a few other PhACs, as well as their confirmation by undertaking conventional degradation assays. Computer-aided techniques and their integration into experimental catalysis could offer a new dimension to archive goals as a green non-testing method of degradation of unexplored PhACs from the environment. Most often computer-aided degradation prediction flow utilizes docking, MD simulation, DFT, homology modeling, and a few other techniques. Such functionality enables insight into laccase binding and catalysis, real-time binding behavior, and a comprehension of the atomic-level degradation process. Further, all this information can be applied to the development

of an engineered protein to have a highly catalytic function against non-degradable PhACs. Several laccase features are anticipated to be enhanced to facilitate environmentally friendly remediation of contaminants in the specified setting. These features include the engineering of microorganisms for high-yield enzyme production, the targeting of the coding gene and the search for its homology in other species for enzymatic production, the development of synthetic enzymes based on their native sequence, and the encouragement of laccase in computational investigations of unexplored PhACs. A comprehensive detail of eco-friendly strategies utilizing laccase for the removal of PhACs from water is critically discussed focusing on their efficiencies, and current limitations to design improved technologies for their lab-to-field applications. Furthermore, the review highlights the broad array of PhACs in water bodies and suggests the scope of a laccase-mediated system for enhanced removal of pharmaceutical residues from water to fulfill the United Nations Sustainable Development Goal (UN-SDG-6) for providing clean potable water for all. Nevertheless, it is anticipated that a few factors will be integrated in the future to address the current challenges:

- ✓ Development and innovation of PhACs detection methods from water resources
- ✓ Screening and easy AMR detection method for identifying inhabitant-resistant microbial species
- ✓ Improve laccase production in native producers for high-yield
- ✓ Deployment of laccase using novel framework i.e. MXenes for scale up the practical applicability in degradation of PhACs

- ✓ Bionanozyme for environmental remediation of PhACs

CRediT authorship contribution statement

AKS: Writing review & editing, figures, table, final draft, revision, Conceptualization. **PA:** Writing review & editing, figures. **DA:** Writing review & editing, figures. **IC:** Writing – review & editing, Validation, Conceptualization. **RFL:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **MB:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Conflict of interest

No conflict of interest exists in this work.

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