

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

3 Anil Kumar Singh ¹, Pedro Abellanas-Perez ², Diandra de Andrades ^{2,3}, Iris Cornet ⁴,
4 Roberto Fernandez-Lafuente ^{2,*} Muhammad Bilal^{5,6*}

5

6 ¹ Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India

7 ²Department of Biocatalysis, ICP-CSIC, C/ Marie Curie 2, Campus UAM-CSIC
8 Cantoblanco, Madrid, Spain

9 ³Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão,
10 Preto, University of São Paulo, Ribeirão Preto 14040-901, SP, Brazil

11 ⁴BioWAVE research group, University of Antwerp, Groenenborgerlaan 171, 2020
12 Antwerp, Belgium

13 ⁵Department of Sanitary Engineering, Faculty of Civil and Environmental
14 Engineering, Gdansk University of Technology, G. Narutowicza 11/12 Str., 80-233
15 Gdansk, Poland

16 ⁶Advanced Materials Center, Gdansk University of Technology, 11/12 Narutowicza
17 St., 80-233, Gdansk, Poland

18 *Co-corresponding authors: muhammad.bilal@pg.edu.pl (M.B), rfl@icp.csic.es
19 (R.FL)

20 **Abstract**

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

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

44



45 **1. Introduction**

46 A new class of organic pollutants known as pharmaceutically active compounds
47 (PhACs) primarily consists of hormones, antibiotics, antifungal agents,
48 antidepressants, anti-epileptics, hypoglycemic medications, analgesics, beta-
49 blockers, and nonsteroidal anti-inflammatory medicines (NSAIDs) [1, 2]. The
50 potential application of PhACs is widely employed in agriculture, aquaculture, animal
51 husbandry, and human healthcare globally [1]. PhACs are not used by organisms
52 substantially, and most of them are flushed out of the body in their parent form
53 before being metabolized, the vast majority of these compounds end up in the
54 environment or wastewater treatment facilities (WWTPs) [1]. The concentration of
55 PhACs in water systems has been reported in a varying range from ng L^{-1} to $\mu\text{g L}^{-1}$
56 [3, 4]. Their existence in aquatic ecosystems is a consequence of the direct release
57 of WWTP effluents into water bodies [5]. These PhACs are present in both the
58 influent and effluent wastewater, but they might also be detected in other kinds of
59 surface water including freshwater and marine environments as well as in
60 groundwater via effluent leachates [6]. Over the last decade, the increase in the
61 global population has led to elevated drug use and, therefore, a significant excretion
62 of both the drug and its metabolites [5]. The scarcity of information concerning the
63 sources, transport, and accumulation of PhACs in aquatic ecosystems has resulted
64 in uncertainty in associated potential risks they may exhibit during their interactions
65 with aquatic bodies. On a global scale, these compounds are among the most
66 critical emerging contaminants (EC) found in water sources [5]. Consequently,
67 PhACs have devastating consequences on aquatic organisms, since exposure to
68 them has negative reproductive effects in the early life stages of several species [7-
69 10]. As a result, this issue requires serious consideration as a way to address it



70 promptly. A wide range of physical, physicochemical, and chemical methods
71 including membrane processes, advanced oxidation processes (e.g., ozonation, UV
72 photolysis or UV/H₂O₂) adsorption are often employed to remove pharmaceutical
73 contamination from water [11-13]. Nevertheless, these methods have several
74 disadvantages, including the generation of toxic by-products in advanced oxidation
75 processes, the disposal challenges associated with concentrated waste in
76 membrane processes, and the cost of operation, etc. [14]. For instance, the high
77 expense of the reagents (such as H₂O₂) and energy required to generate O₃ or UV
78 light is one major drawback of advanced oxidation processes [15]. Compared to
79 physicochemical or other non-biological processes, biological processes particularly
80 enzymatic remediation offer several benefits including; In addition, including
81 advantages of environmental friendliness, high efficiency, broad substrate specificity,
82 less toxic need less energy, and not required for any extra nutrients to operate
83 designed experiments [14, 16].

84 Laccases (EC 1.10.3.2) are multicopper-containing oxidoreductases that are
85 potent biocatalysts and have been exploited in the bioremediation of a broad range
86 of pollutants including a wide range of PhACs [17-24]. Laccases can oxidize diverse
87 phenolic and non-phenolic contaminant compounds employing molecular oxygen as
88 an electron acceptor, with water as the only by-product [25, 26]. This is a clear
89 advantage when compared to other enzymes (peroxidases or oxidases) with oxidant
90 potential that could be used for similar functions, as both have H₂O₂ in their catalytic
91 cycle (Fig. 1) [27]. Peroxidases have been proposed in many instances to perform
92 this bioremediation function, but these enzymes require the use of H₂O₂ as an
93 oxidant cosubstrate [28-31]. This means that the actual use of these enzymes in
94 bioremediation may be hindered, as it may not be easy to add this dangerous



95 reagent to the environment due to its negative biological effects, although this fact
96 used to be not considered. This may be stressed if the environment is as large as
97 the ocean. Oxidases are other alternatives to destroy contaminant compounds [28,
98 32, 33]. They do not require the addition of H₂O₂ as a co-substrate (Fig.1). As
99 laccases do, they can utilize molecular oxygen as an oxidant. However, in their
100 catalytic cycle, the FAD-dependent oxidases produce hydrogen peroxide as a by-
101 product [27, 34, 35]. Laccases are extensively prevalent in the environment (e.g.,
102 plants, insects, fungi, and bacteria)) [17]. Such prevalence has been caused by their
103 multiple physiological roles. For example, within plants, they are engaged in lignin
104 biosynthesis (polymerization from monolignols), while bacterial and fungal-derived
105 laccase (Table 1) are most often deployed systems involved in the degradation of
106 lignin, phenolic, and pharmaceuticals [36-38]. The catalytic reaction of laccase
107 triggers the cleavage of each specific compound in a unique way, i.e., C-C cleavage,
108 oxidation, or alkyl-aryl cleavage; the same pattern is utilized to break down both β -1
109 and β -O-4 dimers of lignin compounds [39, 40]. Laccases catalyze oxidative coupling
110 or bond breakage of target molecules by one-electron oxidation and
111 subsequent radical formation [41]. Such attribute of laccase may oxidize several
112 phenolic and nonphenolic substances owing to its broad substrate specificity [42,
113 43]. Based on distinct spectroscopic features, the four copper atoms in a typical
114 laccase molecule are classified as Type 1 (T1), Type 2 (T2), and binuclear Type 3
115 (T3) Cu sites [44]. The four copper ions in the resting enzyme are in the +2 oxidation
116 state [44]. Plant and bacterial laccases typically have low redox potentials (e.g., 0.43
117 and 0.46 V for *Rhus vernicifera* and wild-type *Bacillus subtilis* CotA laccases,
118 respectively), whilst, white-rot fungi exhibit comparatively higher redox potential
119 (0.720–0.790 V) [41]. A rising global issue in recent years has been the presence of



120 ECs, which include a variety of PhACs, in both the environment and WWTPs [45,
121 46]. PhACs and their metabolites are usually discharged into the environment by
122 urban agricultural discharge, hospital effluent, and treated and untreated industrial
123 wastewater [45, 46]. Hospitals primarily discharge PhACs into the environment as a
124 consequence of patient excretion and the improper disposal of remaining
125 medications [46]. A schematic representation of PhACs emergence from diverse
126 sources and their prevalence in water matrices is portrayed in Fig. 2. Several PhACs
127 are not eliminated through WWTPs and are released into the environment either in
128 the form of non-degraded or in metabolites [47]. Despite this, the WWTPs are not
129 initially designed with the distinct objective of eliminating these hazardous chemicals
130 [48]. The magnitude of toxicological hazards of such PhACs has been explained
131 thoroughly in a separate section. For example, antibiotics such as sulfamethoxazole
132 can induce genetic alterations and long-term effects in aquatic animals, even at low
133 concentrations [49-51]. Conversely, analgesics such as paracetamol can increase
134 the risk of asthma, liver damage, and kidney cancer in humans [52, 53]. Laccase-
135 based biocatalysts are well-suited to expediting the development of environmentally
136 friendly, sustainable, and efficient industrial deployments to mitigate the
137 aforementioned EC from water matrices [23]. However, the affordability and
138 effectiveness of the enzymes restrict the widespread use of laccases. Considerable
139 efforts have been undertaken to enhance the production of substantial quantities of
140 laccases at a low cost by the use of recombinant organisms or the identification of
141 natural hypersecretory strains [54]. Enzyme activity and stability can be improved by
142 employing appropriate immobilization techniques, including the use of bionanozyme
143 methods, as discussed below [55]. The immobilization of enzymes without
144 compromising their activity offers a potential solution to these issues and enables



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

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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
<i>Aureobasidium pullulans</i> 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]
<i>Pleurotus ostreatus</i> POXA3b	ABTS	74 μM		158 333	2.1 × 10 ⁹	pH 3.6, 25 °C	[65]
<i>Pleurotus ostreatus</i> POXA3a	ABTS	70 μM		73 333	1.0 × 10 ⁹	pH 3.6, 25 °C	[65]
<i>Lentinus</i> sp.	ABTS	65 μM		3382	5.2 × 10 ⁷	pH 2.5, 70 °C	[65]
<i>Trametes hirsuta</i>	ABTS	41 μM		196	4.8 × 10 ⁶	pH 5.0, 25 °C	[65]
<i>Rigidoporus lignosus</i>	ABTS	200 μM		1360	6.8 × 10 ⁶	pH 3.0, 25 °C	[65]
<i>Meripilus giganteus</i>	ABTS	17 μM		546	3.7 × 10 ⁷	pH 3.0, 30 °C	[65]
<i>Agaricus bisporus</i>	ABTS	134 μM		7885	5.9 × 10 ⁷	pH 3.0, 65 °C	[65]
<i>Trametes versicolor</i>	ABTS	38 μM		26 803	6.0 × 10 ⁸	pH 3.0, 65 °C	[65]
<i>Tricholoma mongolicum</i>	ABTS	2 μM		1480	6.4 × 10 ⁸	pH 4.5, 30 °C	[65]
<i>Yersinia enterocolitica</i> strain 7	ABTS	675 μM	0.125 μmol/ml/min			pH 9.0 and stable at 70 °C	[66]
<i>Aspergillus niger</i>	ABTS					pH 4.5, 45 °C	[19]
<i>Coriolus brevis</i>	ABTS	0.02 mM			7.2 × 10 ⁶	Optimal reaction pH 2.5 30–90 °C	[67]
<i>Bacillus</i> 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]

168 **2. Routes, occurrence, and associated hazards of pharmaceutical**
169 **contaminants**

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



193 bodies. A variety of negative impacts, that include genotoxic, teratogenic,
194 reproduction-disturbing, acute cytogenotoxic, oxidative stress, endocrine disruption,
195 growth inhibition (EC₅₀), deformations, and others, have been observed in aquatic
196 organisms as a result of pharmaceutical contamination [70, 77-79]. Detailed
197 toxicological hazards of pharmaceuticals on aquatic organisms have been listed in
198 Table 2. Gutiérrez-Noya et al. [78] reported that ibuprofen induces teratogenesis,
199 and oxidative stress, and alters embryonic development in *Cyprinus carpio*.
200 Subsequently, at concentrations between 1.5 and 11.5 µg L⁻¹, ibuprofen was capable
201 of inducing alterations to embryonic development, teratogenic effects, and oxidative
202 stress in oocytes and embryos of *Cyprinus carpio*. As a conclusion, the major
203 embryonic development abnormalities and teratogenic consequences were delayed
204 hatching, hypopigmentation, pericardial edema, yolk deformation, and
205 developmental delay in *Cyprinus carpio*.

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

217

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 <i>Caenorhabditis elegans</i>	[81-83]
Norfloxacin	Antibiotic	Reproductive toxicity on <i>Caenorhabditis elegans</i>	[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 <i>Vibrio fischeri</i> and <i>Daphnia Magna</i>	[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]

219

220 3. Structural and chemical aspects of laccases

221 As stated above, laccases belong to the protein superfamily known as multicopper
222 oxidases [102-104]. Enzymes of this type exhibit catalytic activity in the oxidation of a
223 wide variety of compounds, notably those with phenolic moieties. Furthermore, these
224 enzymes are not only important in numerous biological processes but also relevant
225 in the area of biotechnology [105, 106]. The catalytic cycle of laccase may be
226 improved by employing a mediator system to degrade the specific contaminant
227 (Fig.3). The molecular structure of laccases is characterized by a high abundance of
228 beta components in its protein structure, distinguishing them from peroxidases [105,
229 107, 108]. Although laccases are structurally complicated, they are typically
230 monomeric enzymes [105]. The Protein Databank (<https://www.rcsb.org>) contains a
231 wide range of laccases structures from many sources, characterized by their crystal
232 structures solved by different methods containing diverse structural architects [109,
233 110]. For instance, the laccase from *Thermus thermophilus* (PDB: 6TYR) comprised
234 439 amino acids in a single chain with a molecular weight of around 49.45 kDa [109,
235 111, 112]. Likewise, a fungal laccase, from *Cerrena maxima* origin (PDB: 2H5U),
236 comprises 499 amino acid residues in a single chain with a molecular weight of
237 around 55.67 kDa [109, 113]. A laccase from plant origin (*Zea mays*), PDB: 6KLJ
238 comprises 550 amino acid residues in a single chain with a molecular weight of

239 63.91 kDa [109, 114, 115]. The laccase from *Drosophila erecta*, with accession no:
240 XP_001981736.2, translated to have 677 amino acid residues with a calculated
241 molecular weight of 77.56 kDa [116]. The laccase from *Trametes versicolor* (PDB:
242 1KYA) contains 499 amino acid residues distributed in four chains (A, B, C, and D)
243 with a molecular weight of 221.72 kDa [117]. The constituent amino-acid residues of
244 no single laccase are identical to those of others. Detailed quantitative information on
245 protein secondary structure elements of laccases from different sources have been
246 portrayed in Fig. 4. However, it is essential to note that the use of bacterial and
247 fungal laccases has been primarily employed in the degradation of PhACs; however,
248 comparison to different origin may provide the structural similarity in few aspects. A
249 closer look at the multicopper binding site of laccase with an active site of copper
250 binding is portrayed in Fig. 5.

251 **4. Chemical scheme and catalytic functionalities of laccases**

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

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

275 **5. Laccase immobilization for enhancing catalytic potential towards** 276 **pharmaceutical degradation**

277 Compared to laccase in its free state, the immobilization of laccase may result in
278 improved stability concerning storage, temperature, and pH. Enzyme immobilization
279 consists of the location of the enzyme in a confined space [121]. This started as a
280 technology intended to simplify the capture and reutilization of these biological and
281 initially very expensive biocatalysts [122, 123]. The recycling, operational stability,
282 and resistance to application conditions of laccases are all enhanced when the
283 enzymes are immobilized [124]. Some examples of immobilization methods
284 encompass entrapment, adsorption, covalent binding, self-immobilization, and so on
285 has portrayed in Fig.7. In some instances, immobilized laccases can withstand high
286 temperatures, storage, and reusability in a better way than their free counterparts.
287 The control of enzyme immobilization requires a fine control of the process, and
288 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



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



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

346 Taheran et al. [144] reported the Immobilization of laccase onto a nanofibrous
347 membrane for the degradation of chlortetracycline (CTC), carbamazepine (CBZ),
348 and diclofenac (DCF) residues in water. The results indicated that the immobilized
349 laccase exhibited superior pH, temperature, and storage stability in comparison to
350 the free laccase. Additionally, it maintained over 17% of its initial activity after 10
351 cycles of ABTS oxidation, indicating that the enzyme's reusability was improved. The
352 degradation efficiency of three pharmaceutical compounds in batch experiments was
353 72.7% (DCF), 63.3% (CTC), and 48.6% (CBZ) after 8 hours of reaction when
354 immobilized laccase was employed. Al-sareji et al. [24] reported laccase
355 immobilization on activated carbon derived from pomegranate peels to remove
356 diclofenac, amoxicillin, carbamazepine, and ciprofloxacin from water and
357 wastewater. The subsequent study showed pomegranate peels were successfully
358 used as an adsorbent and enzyme carrier for the removal of emerging contaminants,
359 even from complex sample matrices. The removal of contaminants from effluent was
360 completed in five cycles, while it was extended to six cycles for water.

361

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	<ul style="list-style-type: none"> -Easy modification with different functional groups -Electrical conductivity -Good mechanical strength -Considerable adsorption capacity 	<ul style="list-style-type: none"> -Possibility of contamination -High production cost Toxicity
Magnetic support	<ul style="list-style-type: none"> -Easy surface modification -Good magnetic responsiveness -Easy separation and good reusability 	<ul style="list-style-type: none"> -Susceptible to acidic and oxidative conditions -Agglomeration problem
Inorganic support	<ul style="list-style-type: none"> -Low cost -Low impact on the environment -High pH and thermal stability -High mechanical strength 	<ul style="list-style-type: none"> -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	<ul style="list-style-type: none"> -Abundant functional groups and easy modification -High enzyme loading -Strong enzyme-support binding -Pore diameter may be chosen -Large specific surface area 	<ul style="list-style-type: none"> -High cost -Non-renewable -Complex synthesis process
Natural organic support	<ul style="list-style-type: none"> -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 	<ul style="list-style-type: none"> -Possibility of bacterial degradation -Low mechanical rigidity
COFs	<ul style="list-style-type: none"> -Low density -Large surface area -Regular and orderly pore structure -Structural pre-designability 	<ul style="list-style-type: none"> -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

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



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



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



465 This can include copolymers, crosslinked enzyme crystals (CLECs) or aggregates
466 (CLEAs), nanoflowers, sol-gels, crystals coated with enzymes, enzymes trapped in
467 polymer-formed ex novo (e.g., calcium alginate or lentikats beads), etc. [133-135,
468 137, 160-173]. Finally, the third class of immobilization protocols will be formed by
469 those where a preexisting solid is utilized as a matrix for the enzyme immobilization
470 (the immobilization may be via covalent bonds or physical interactions) [138-140,
471 174]. These supports may be nonporous (nanomaterials, membranes) or porous
472 materials, and each of them may have advantages and drawbacks, depending on
473 the specific enzyme, reactor, and application [141]. One point usually not considered
474 in immobilization is the possibility of using immobilization techniques where the
475 particle of the biocatalyst can promote the partition of the substrate (Fig. 10) [150].
476 The increase or the reduction of the concentration of the substrate in the enzyme
477 environment may be interesting, depending on the circumstance. For example, in
478 cases where the contaminant substance to be eliminated is at a very low
479 concentration in the media, far below the enzyme saturation concentration, and that
480 must be maintained that way to prevent damage to the environment, the enzyme
481 performance will be reduced. In this instance, it may be interesting to use a
482 support/modification of the enzyme with polymers where the substrate can become
483 favorably partitioned, and that way to have in the enzyme environment a higher
484 substrate concentration that permits the enzyme to exhibit maximum activity even
485 when the external substrate concentration may be very low (Fig. 10A). In
486 contraposition, if the enzyme may be exposed to occasional high concentrations of
487 some deleterious reagent (the substrate itself or some other component of the
488 medium with negative effects on enzyme stability) (Fig. 9), a partition from this
489 reagent away from the biocatalyst particle may promote positive effects for the



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



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

525 Masjoudi et al. [145] reported the laccase immobilization on polyvinylidene
526 fluoride nanocomposite with multi-walled carbon nanotubes to be employed in the
527 removal of carbamazepine and diclofenac, which exhibited the removal efficiencies
528 of 27% in 48 h for carbamazepine and 95% in 4 h for diclofenac. Al-sareji et al. [24]
529 reported laccase immobilization to remove diclofenac, amoxicillin, carbamazepine,
530 and ciprofloxacin from water and wastewater. Taheran et al. [144] reported covalent
531 immobilization of laccase onto the nanofibrous membrane for degradation of
532 chlortetracycline, carbamazepine, and diclofenac. It was able to exhibit 72.7%,
533 63.3%, and 48.6% degradation efficiency for chlortetracycline, carbamazepine, and
534 diclofenac respectively, after 8 h of reaction.

535 **7. Deployment of the laccase-biocatalyst system in degrading a wide array of** 536 **pharmaceutical compounds, and toxicity reduction**

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



540 resulted in concerns about water and environmental contamination [184-186]. Most
 541 studied toxicological impacts of PhACs have been reported in Table 6.
 542 Consequently, these pollutants often evade treatment facilities and permeate further
 543 into natural water sources such as groundwater, rivers, and water bodies, ultimately
 544 contaminating drinking water supplies [187, 188]. As a result, there has been a rise
 545 in the study and implementation of biological wastewater treatment techniques with
 546 the purpose of PhACs removal. In recent years, laccase has been deployed in the
 547 degradation of diverse PhACs [37, 189-194]. A few examples of PhAC degradation
 548 by applying laccase in free and immobilized form have been explained in detail in
 549 subsequent sections. Recently reported laccase-assisted PhACs degradation is
 550 summarized in Table 6. The effective function of laccase for lowering toxicity and the
 551 degradation of pharmaceuticals is evident in observations from recent studies [195,
 552 196]. Laccase-based pharmaceutical degradation has been recognized as an
 553 economically viable method for the complete biotransformation of antibiotics from
 554 aquatic media [196]. Furthermore, the agar-diffusion method demonstrated that
 555 biodegraded products were non-toxic and promoted the growth of *Staphylococcus*
 556 *aureus* and *E. coli* [195]. Feng et al. [197] reported the transformation of atenolol
 557 (ATL) by the native laccase from *Trametes versicolor* in an aqueous solution. In a
 558 subsequent study, the toxicity of ATL and TEMPO mixtures was significantly reduced
 559 through laccase treatment. The aforementioned findings suggest that the reduction
 560 of pharmaceuticals by laccase into less toxic transformed compounds is a
 561 sustainable and eco-friendly method.

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

Country wise geo-location	Pharmaceutical compound	Concentration	Wastewaters/sources	Reference
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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		
Saudi Arabia	Trimethoprim	0.05-4.8 µg L ⁻¹	WWTP-1 influent	[198]



	Ciprofloxacin	7.1-711.3 $\mu\text{g L}^{-1}$		
	Diclofenac	0.4-45.3 $\mu\text{g L}^{-1}$		
	Acetaminophen	46.4-943 $\mu\text{g L}^{-1}$		
	Ofloxacin	0.9-88.2 $\mu\text{g L}^{-1}$		
	Metformin	3.1-587.9 $\mu\text{g L}^{-1}$		
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 $\mu\text{g L}^{-1}$	Hospital wastewater	[90]
	Ofloxacin	198 $\mu\text{g L}^{-1}$		
	Ciprofloxacin	228 $\mu\text{g L}^{-1}$		
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 $\mu\text{g L}^{-1}$		
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 $\mu\text{g L}^{-1}$	Urban wastewater	[90]
	Gemfibrozil	2.7–3.2 $\mu\text{g L}^{-1}$		
	Carbamazepine	0.17–0.19 $\mu\text{g L}^{-1}$		
	Ibuprofen	6.4–19.0 $\mu\text{g L}^{-1}$		
France	Cyclophosphamide	0.5–0.8 $\mu\text{g L}^{-1}$	Hospital wastewater	[90]
	Sulfamethoxazole	12.3–33.5 $\mu\text{g L}^{-1}$		
	Atenolol	1.6–6.5 $\mu\text{g L}^{-1}$		
South Africa	Aspirin	118 \pm 0.82 $\mu\text{g L}^{-1}$	Domestic, hospital, commercial, and industrial wastewater	[90]
	Diclofenac	22.3 \pm 0.63 $\mu\text{g L}^{-1}$		

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
<i>Trametes versicolor</i>	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]
<i>Trametes versicolor</i>	Immobilization using electrospun materials	Tetracycline	pH 5, 25 °C	100% and 94% for covalently bonded and encapsulated laccase	[201]
<i>Aspergillus</i> species	Immobilized on zeolitic imidazolate frameworks	Carbamazepine	pH 7.0, 20–70 °C	~92%	[146]
<i>Aspergillus</i> species	Immobilized on a zeolitic imidazolate framework	Diclofenac and norfloxacin	pH 6.4	93.9 and 95.1%	[202]
<i>Trametes hirsuta</i>	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]
<i>Trametes versicolor</i>	Immobilized on date stones	Ketoprofen and aspirin	pH range 2–7.5, 25 °C	Complete removal within 4 h of treatment	[203]
<i>Pleurotus ostreatus</i> and <i>Lentinus sajor-caju</i>	Free	Sulfamethoxazole	pH 5.0, 25 °C	Approximately 100 % of SMX degradation was attained in 30 min	[196]



<i>Pleurotus florida</i>	Free	Ciprofloxacin and norfloxacin	pH 4.5, 30 °C	Ciprofloxacin (86.12–75.94%) and norfloxacin (83.27–65.94%) was achieved	[195]
<i>Trametes versicolor</i>	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]
<i>Pleurotus ostreatus</i>	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	
<i>Trametes versicolor</i>	Free	Doxorubicin	pH 7, 30 °C	Reduction of 41.4% doxorubicin toxicity	[192]
<i>Trametes versicolor</i>	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 (V_{max}) 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

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

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

598

599 **7.2 Degradation of analgesics/NSAIDs**

600 Analgesics, which include both opioid and non-opioid formulations, function as
601 pharmaceutical agents that alleviate pain and that may be found in wastewater [211,
602 212]. Laccases have undergone evaluation against nonsteroidal anti-inflammatory
603 drugs, including aspirin and ketoprofen [14, 203]. The excessive use of aspirin
604 (acetylsalicylic acid), an anti-inflammatory drug commonly prescribed for the
605 treatment of pain and fever, has become a significant environmental pollution
606 concern. This has detrimental consequences for aquatic organisms, which include
607 reproductive and fetal development [14]. Multiple research studies have shown the
608 effectiveness of free laccases in the degradation of NSAIDs [189, 213-215].



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

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



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

646

647 **7.3. Degradation of antibiotics**

648 Antibiotics are employed in livestock farming and human and veterinary medicine,
649 thus constituting one of the most widely utilized classes of medications worldwide
650 [217-220]. Antibiotics that do not undergo decomposition are persistent and get into
651 the environment. Furthermore, the primary challenge with antibiotics is the
652 emergence and spread of resistant bacteria, which has been rendered easier
653 considering the present situation [221]. Antibiotics are not effectively removed either
654 by conventional water treatment processes or advanced treatment methods;
655 although advanced remediation methods are more efficient, they come with
656 drawbacks, including high costs and resulting in secondary pollution [222, 223]. As
657 "green and sustainable biocatalysts" for antibiotic degradation, laccases have proven
658 great potential for the degradation of diverse classes of antibiotics [224]. The primary



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

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

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

679
680 Ouyang et al. [229] pointed out the effective removal of sulfonamides and
681 tetracycline residues by the laccase-mediator system using a novel laccase from
682 *Lysinibacillus fusiformis*. A novel laccase from *L. fusiformis* (Lyfu-Lac) revealed
683 promising removal effectiveness onto sulfonamides and tetracycline residues in the



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

689 Wang et al. [230] reported the elimination of tetracyclines in seawater by a
690 laccase-mediator system. Laccase was utilized from *Aspergillus* sp. To degrade
691 tetracyclines (TCs) in coastal seawater at environmentally significant concentrations
692 (ngL^{-1} - μgL^{-1}), mediators that incorporated distinct oxidation mechanisms were
693 combined. The enzymatic structure of laccase was altered by the elevated salinity
694 and alkalinity of seawater, leading to a decreased affinity for the substrate (K_m of
695 $0.0556 \text{ mmolL}^{-1}$) of laccase in seawater compared to buffer (K_m of $0.0181 \text{ mmolL}^{-1}$).
696 Despite the decline in stability and activity of laccase when exposed to seawater, it
697 was possible to completely degrade TCs in seawater within two hours using laccase
698 at a concentration of 200 U L^{-1} coupled with a laccase/syringaldehyde (SA) ratio of 1
699 U:1 μmol , even at initial concentrations of less than $2 \mu\text{gL}^{-1}$. Sarnthima et al. [231]
700 molecular insight of sulfamethoxazole degradation using laccase from *Streptomyces*
701 sp. CS29. The activity of laccase was shown to be enhanced by 10 and 20 mM Ca^{2+} ,
702 20 mM Zn^{2+} , and 10 mM K^{+} . Based on the results of the following investigation, the
703 best pH for breaking down sulfamethoxazole was 3.0, and the degradation rate was
704 97.90%.

705

706 **7.4 Degradation of antiepileptic agents**

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



710 flocculation, coagulation, and chlorination processes [235]. That way, biocatalytic
711 degradation utilizing enzymes such as laccases presents a potentially effective
712 strategy for the complete removal of CBZ from water matrices [214, 235-237]. The
713 ineffectiveness of recent laccase-based investigations to degrade CBZ was
714 attributed to the electron-withdrawing group amide present in the structure of CBZ
715 [235]. This functional group rendered CBZ strongly electron-deficient and reduced its
716 laccase oxidation potential [235]. Application of redox mediators, with a more
717 suitable potential, including ABTS and HBT, can augment the oxidation capacity of
718 laccase to cope with this concern [235].

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

732 Naghdi et al. [238] reported the removal of CBZ from immobilized laccase on
733 oxygen-functionalized nanobiochars. The impact of applying HCl, H₂SO₄, HNO₃, and
734 their mixtures to oxidize nanobiochar, a carbonaceous material generated through



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

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

760 **7.5 Beta blocker degradation**

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

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

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

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

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

798 **8. Laccase mediator system to enhance the catalytic process for a diverse** 799 **range of pharmaceuticals**

800 Laccase has the ability to catalyze the oxidation of numerous organic compounds.
801 Despite the employment of molecular oxygen, it is considered a green
802 environmentally friendly, cost-efficient, and safe enzyme catalyst
803 [245]. Nevertheless, there are still some issues that need to be resolved when
804 laccase is explicitly employed in environmental remediation. Initially, a significant
805 number of non-phenolic substrates were unable to directly bind to laccase. Secondly,
806 the redox potential of the majority of laccases (0.5-0.8 V) seemed insufficient to
807 oxidize high-potential phenolic compounds and other complex non-phenolic
808 compounds [246]. Schematic illustration of the laccase-mediator model, and its

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

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



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

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

855

856 **9. Computational-aided pre-screening-based approaches for the degradation** 857 **of unexplored pharmaceuticals**



858 Extensive research has been conducted on the vital role of laccases in the
859 degradation of xenobiotics, consisting of phenols, anilines, and PhACs [43, 179, 219,
860 251, 252]. Despite its broad substrate specificity, it enables numerous screening
861 opportunities for the sustainable removal of PhAC in a sustainable way [14, 253,
862 254]. Despite advancements in numerous approaches to pollutant removal, which
863 include enzyme-conjugated nanocomposites and immobilized enzymes, a significant
864 concern persists concerning their complete degradation and eventual fate in the
865 environment, and left over the toxicity after experimental operation [63, 255, 256].
866 These concerns pertain to the fate of completely transformed metabolites, the active
867 site amino acid responsible for catalysis, and molecular interaction among amino
868 acids and pollutants, which remain unsolved in conventional remediation methods
869 [61, 63, 107, 257]. Computational degradability prediction of concerned PhACs with
870 a possible enzyme is a robust approach that could deal with inadequacies in a joint
871 effort by validating such outcomes through a conventional real-time degradation
872 assay under controlled settings [60, 63, 258, 259]. Such aforementioned
873 degradability predictions include docking, MD-Simulation, and degradation pathways
874 prediction that have been employed to unravel the degradation process at the atomic
875 level [60, 108, 260]. Docking methods are often used to identify the best
876 conformational state of the docked complex (enzyme-pollutant), as well as the
877 binding contact between the pollutant and the enzyme's active site [57, 61, 107, 108,
878 261-263]. Enzyme-pollutant docking analyses facilitate the visualization and
879 estimation of the most frequent amino acid residues involved in pollutant binding, as
880 well as the straightforward determination of chemical bonds between the
881 corresponding enzyme-pollutants [107, 261]. However, docking alone is insufficient
882 for exhibiting the actual catalytic activity of enzyme-contaminants in real-time [61,



883 62]. An MD simulation may be undertaken to observe the real-time conformational
884 behavior of enzyme and pollutant bound complex using an appropriate time-scale
885 simulation run under a specific build model system (i.e. NPT or NVT) [61]. MD
886 simulation offers valuable information on how enzymes or proteins interact with
887 pollutants (ligands), focusing on the chemical bond formation and the role of active
888 site residues in the degradation process, including the post-simulation energy
889 analyses, and system equilibrium state [61, 260]. Such functionality could be
890 implemented to predict the degradability and catalytic potential of an enzyme toward
891 targeted PhAC. Nonetheless, protein engineering techniques could be used to
892 improve the enzyme's capability to better binding and catalytic attributes [264-266].
893 The proposed computational framework for the binding and chemical functionalities
894 of PhACs in the context of the degradation mechanism, which is based on laccase,
895 can be seen in Fig. 13. A clear binding amino-acid residues and Gibbs free energy
896 (ΔG) assessment can be observed through above explanatory illustration. In the
897 context of the above functionalities, physico-chemical properties of laccase from
898 varying origins may or may not be comparable, which ultimately influences the
899 binding and degradation of target contaminants under specific environmental
900 conditions. Consequently, it is of the utmost importance to comprehend the various
901 parameters of physico-chemical properties. Therefore, diverse physicochemical
902 properties of laccases have been summarized in Table 7.

903 Nawaz et al. [267] reported bioremediation potential of laccase from *Bacillus*
904 *ligniniphilus* L1 in set of 18 antibiotic degradation (penicillin, levofloxacin,
905 cephalosporin, tobramycin, linezolid, clindamycin, metronidazole, chloramphenicol,
906 nitroimidazole, fosfomicin, tetracyclines, rifamycin, vancomycin, daptomycin,
907 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)
<i>Trametes versicolor</i>	1KYA	499	C ₂₃₉₉ H ₃₆₀₀ N ₆₃₈ O ₇₂₉ S ₉	53331.35	45	20	4.69	26.88	81.34	-0.028
<i>Streptomyces carpinensis</i>	8AIP	293	C ₁₄₀₇ H ₂₁₄₈ N ₄₁₂ O ₄₂₇ S ₁₆	32180.00	37	37	5.95	41.39	63.45	-0.495
<i>Streptomyces viridosporus</i>	3TBB	313	C ₁₄₉₈ H ₂₂₈₀ N ₄₄₄ O ₄₅₆ S ₁₁	34157.94	41	31	6.16	34.54	60.73	-0.657
<i>Thermus thermophilus</i> 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
<i>Coriolopsis trogii</i>	2HRH	496	C ₂₃₈₂ H ₃₆₀₄ N ₆₃₆ O ₇₂₉ S ₉	53103.18	48	23	4.83	36.55	82.80	-0.082
<i>Cerrena caperata</i>	4JHU	496	C ₂₃₈₇ H ₃₆₀₉ N ₆₄₃ O ₇₂₉ S ₁₁	53330.44	47	23	4.87	35.38	80.83	-0.127
<i>Streptomyces griseoflavus</i>	7PEN	322	C ₁₅₁₁ H ₂₃₁₉ N ₄₅₁ O ₄₆₂ S ₁₃	34611.56	37	27	6.06	33.75	66.96	-0.433
<i>Melanocarpus albomyces</i>	3FU7	559	C ₂₇₆₄ H ₄₁₅₃ N ₇₅₉ O ₈₃₁ S ₁₅	61791.87	62	34	4.91	32.71	78.59	-0.325
<i>Trametes maxima</i>	2H5U	499	C ₂₃₈₄ H ₃₅₇₁ N ₆₅₃ O ₇₃₄ S ₇	53347.93	38	21	5.26	30.41	73.51	-0.207



<i>Coriolopsis gallica</i>	4A2E	496	C ₂₃₇₁ H ₃₅₇₉ N ₆₃₁ O ₇₂₂ S ₉	52763.83	45	21	4.84	31.42	83.25	-0.023
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~~927~~ Physicochemical properties have been predicted using the ProtParam – Expasy tool by utilizing amino acid sequences from corresponding PDB IDs.

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939 **10. Concluding remarks and future outlook**

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

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



989 of an engineered protein to have a highly catalytic function against non-degradable
990 PhACs. Several laccase features are anticipated to be enhanced to facilitate
991 environmentally friendly remediation of contaminants in the specified setting. These
992 features include the engineering of microorganisms for high-yield enzyme
993 production, the targeting of the coding gene and the search for its homology in other
994 species for enzymatic production, the development of synthetic enzymes based on
995 their native sequence, and the encouragement of laccase in computational
996 investigations of unexplored PhACs. A comprehensive detail of eco-friendly
997 strategies utilizing laccase for the removal of PhACs from water is critically
998 discussed focusing on their efficiencies, and current limitations to design improved
999 technologies for their lab-to-field applications. Furthermore, the review highlights the
1000 broad array of PhACs in water bodies and suggests the scope of a laccase-mediated
1001 system for enhanced removal of pharmaceutical residues from water to fulfill the
1002 United Nations Sustainable Development Goal (UN-SDG-6) for providing clean
1003 potable water for all. Nevertheless, it is anticipated that a few factors will be
1004 integrated in the future to address the current challenges:

- 1005 ✓ Development and innovation of PhACs detection methods from water
1006 resources
- 1007 ✓ Screening and easy AMR detection method for identifying inhabitant-resistant
1008 microbial species
- 1009 ✓ Improve laccase production in native producers for high-yield
- 1010 ✓ Deployment of laccase using novel framework i.e. MXenes for scale up the
1011 practical applicability in degradation of PhACs



1012 ✓ Bionanozyme for environmental remediation of PhACs

1013 **CRedit authorship contribution statement**

1014 **AKS:** Writing review & editing, figures, table, final draft, revision,
1015 Conceptualization. **PA:** Writing review & editing, figures. **DA:** Writing review &
1016 editing, figures. **IC:** Writing – review & editing, Validation, Conceptualization.
1017 **RFL:** Writing – review & editing, Validation, Supervision, Project administration,
1018 Funding acquisition, Conceptualization. **MB:** Writing – review & editing, Validation,
1019 Supervision, Project administration, Funding acquisition, Conceptualization.

1020 **Conflict of interest**

1021 No conflict of interest exists in this work.

1022 **Acknowledgment**

1023 The financial support from the National Science Centre, Poland under the Research
1024 Grant number UMO-2023/49/B/ST8/02271 is graciously acknowledged. RFL thanks
1025 Ministerio de Ciencia e Innovación and Agencia Estatal de Investigación (Spanish
1026 Government) (project PID2022-136535OB-I00).

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