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1           **Engineering biocatalytic material for the remediation of pollutants: A**  
2   **comprehensive review**

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21 **Short title:** Biocatalysts for remediation of pollutants

22 **ABSTRACT**

23 Bioremediation through biotechnological interventions has attracted more attention among  
24 researchers in field of environmental pollution control and abatement. Various cutting-edge  
25 studies in area of protein engineering and synthetic biology offer a new platform for creation  
26 of innovative, advanced biological materials for its beneficial role in environmental pollution  
27 mitigation. Biocatalysis especially receives considerable attention as sustainable approach to  
28 resource recovery from waste along with elimination of pollutants. This paper focuses on  
29 updated developments in engineering of biocatalytic substances which can degrade pollutants  
30 of emerging concern. It also explains various classes of biocatalysts, their mechanisms of  
31 immobilization, and applications in terms of environmental pollutant remediation.  
32 Opportunities and challenges for future research have also been discussed.

33 **Keywords:** Biocatalyst; Protein engineering; Pollutants; Immobilization; Bioremediation

34 **1. Introduction**

35 Various forms of emissions profoundly affect the environment. Manufacturing, human  
36 activities, and agricultural disposals processing make a major part in polluting air, water, and  
37 soil. A subclass of organic compounds alarmingly found in the environment enormously has  
38 labeled as emerging pollutants (EPs). These are otherwise known micropollutants (MPs) or  
39 emerging concern contaminants (Teodosiu et al., 2018; Varjani and Sudha, 2018). Such  
40 micropollutants are identified as processed synthetic chemicals without being tracked or  
41 controlled in most situations. These negatively impact people's health and many other life

42 forms (Sauvé and Desrosiers, 2014; Mohan et al., 2018; Bilal et al., 2019). These are  
43 otherwise called as Persistent organic pollutants (POPs). These POPs include perfluoroalkyl  
44 and polyfluoroalkyl, polycyclic aromatic hydrocarbons (PAHs), pesticides (halogenated  
45 organic compounds), and so on (Daniel et al., 1998; Mandal et al., 2015; Mandal et al., 2018;  
46 Varjani et al., 2019; Kumar et al., 2020). POPs are recognized to always have devastating  
47 health consequences concerning abnormal brain malfunction, growth, metabolic disorders,  
48 hormonal imbalance, etc. (Noakes et al., 2006; Mishra et al., 2019; Femina et al., 2020)  
49 whereas long term exposure may also lead to immunological impacts (Varjani et al., 2020b).

50 A huge number of environmental pollutants like dyes, nitrogen-containing chemicals,  
51 polychlorinated biphenyl compounds (PCBs), plastics, petroleum products, heavy metals,  
52 pesticides, hydrocarbons persist in the ecosystem. They are released from different industrial  
53 sectors and various agricultural resources (Benyahia et al., 2016; Varjani et al., 2017; Kumar  
54 et al., 2018; Rajmohan et al., 2019; Lakshmi et al., 2020). These pollutants are carcinogenic  
55 and highly toxic. In some cases, accumulations of these pollutants become hazardous to the  
56 also flora and fauna living in the environment (Varjani, 2017). Recently pollutant reduction  
57 and depletion are a big concern for environmental science (Do et al., 2020). Originally, waste  
58 produced from different industries was treated by incineration based on high temperature or  
59 by dumping off in a hole as biopile. Due to reduced efficiency, increased cost, and the  
60 formation of other recalcitrant derivatives such approaches were not proven quite successful.  
61 Further, bioremediation came to a picture, which provides a mechanism degradation of these  
62 pollutants by microorganisms (Vidali, 2001; Dzionek et al., 2016; Varjani et al., 2020a).

63 Through the use of microbes as well as their enzymes for pollutant removal is a safe,  
64 efficient, and cheaper process (Bhatnagar et al., 2017; Varjani and Upasani, 2017; Rathna et  
65 al., 2018). Progress in molecular microbiology and recombinant DNA technology can be  
66 made to improve the bioremediation process by plant and microbe genetic modification  
67 (Ramos et al., 2011; Mishra et al., 2020). Many forms of biocatalysts are deeply engaged in  
68 biological treatment, such as hydrolases, oxidoreductases, laccases, and peroxidases (Kadri et  
69 al., 2017). Various microbial sources like fungi (Example: *Pleurotus eryngii*, *Trametes*  
70 *versicolor*), algae (Example: *Chlamydomonas reinhardtii*, *Monoraphidium braunii*), and  
71 bacteria (Example: *Rhodococcus erythropolis*, *Pseudomonas aeruginosa*) were concluded  
72 having a catabolic process for pollutant clean up at pollution sites. Microbial lipolytic  
73 enzymes have gained attention for their ability to catalyse biotransformation reactions of  
74 ester-bond containing compounds eg. conversion of waste into high-energy products like  
75 biofuel and other value-added products via energy-efficient pathways (Kumar et al. 2020).  
76 As reported by Joutey et al. (2013), some microorganisms are capable of degrading  
77 contaminants under laboratory conditions only. This is due to environmental factors  
78 (nutrients, temperature, substrates, and electron acceptors) as they possess major role in  
79 bioremediation and influences biodegradation reactions (Varjani et al., 2019). Xu et al.  
80 (2017), have reported two bacterial strains named *P. aeruginosa* JLC1 and *Acinetobacter* sp.  
81 JLS1 for biodegradation of C16 alkane. They concluded about the temperature-sensitivity of  
82 these strains during the biodegradation process. Moreover, soil texture, occurrence of  
83 pollutants in soil matrix, indirectly affect biodegradation efficiency (Abed et al., 2015).

84 In the present review, groups of biocatalysts used for degradation of an array of pollutants  
85 and how they help in speedy degradation of various toxic pollutants have been summarized.

86 Eco-toxicological assessment for biocatalytic degradation process followed by immobilized  
87 biocatalysts and their effectiveness in removal of pollutants have also been discussed.

## 88 **2. Biocatalysis as a sustainable approach**

89 Various enzyme mechanisms have been used to effectively degrade complex organic  
90 compounds and have demonstrated that the compounds are oxidized and transformed into  
91 simpler intermediates (Chen et al., 2016; Jishao et al., 2019; Chen et al., 2020; Rajmohan et  
92 al., 2020). A relatively recent and convincing area of research is the biological approach,  
93 which uses peroxidases (oxidoreductase group) for the depletion of pollutants. The use of  
94 enzyme-based therapies provides numerous benefits, such as decreased sludge production,  
95 functioning at high and low pollutant concentrations, low energy inputs, and many others,  
96 catalytic research on a wide variety of contaminants (Bilal and Iqbal, 2019). Various studies  
97 have been reported on the biocatalytic removal of major pollutants and have been confirmed  
98 as effective ways in degradation of these compounds in a sustainable manner (Lin et al., 2017;  
99 Gonzalez-Coronel et al., 2017; Rani et al., 2017).

100 While enzyme-mediated cleanup has many benefits, it is important to note that there are some  
101 difficulties, such as the risk of producing hazardous dissolved by-products and the inability to  
102 handle with environmental influences (Zdarta et al., 2018a). Any of such problems can be  
103 overcome by immobilizing that enzyme on various inert surfaces. The examples for various  
104 categories of inert surfaces are silica, metal oxides (e.g. alumina, titania and magnetic iron  
105 oxides II and III), natural polymers (agar, gelatin, alginate, carrageenan, collagen, agarose),  
106 synthetic polymers (polyacrylonitrile, polystyrene, ion exchange resins ) etc. (Daronch et al.  
107 2020).

108 Furthermore, there are several examples of such enzymes effectively insolubilized to  
109 overcome the above-mentioned drawbacks of enzyme reusability and recycling (Alneyadi et  
110 al., 2018; Shao et al., 2019). Peroxidase and Laccase are enzymes commonly used to  
111 bioremediate polluted wastewater. Laccase is suitable for treating wastewater, as it maintains  
112 its operation across a wide range of temperature and pH. Peroxidases have a heme cofactor at  
113 their active sites and possess traces of redox-sensitive cysteine / selenocysteine. Due to the  
114 easy access to their active sites, peroxidase can help to facilitate the removal of many  
115 contaminants from wastewaters (Arca-Ramos et al. 2017). Such enzymes catalyze the  
116 oxidation-reduction of different types of harmful toxins including phenols, cresols, herbicides,  
117 synthetic clothing dyes, pesticides, chlorinated phenols, dioxins, and pharmaceuticals through  
118 assisted biodegradation process (Zdarta et al., 2018b; Muhammad et al., 2019). Although,  
119 biocatalysis is a sustainable approach in pollutant removal, process to produce biocatalyst  
120 requires high cost and some of them are not economical and sustainable. In addition to that  
121 they are unstable and most of them can't withstand several harsh experimental/environmental  
122 conditions (strong acid, high salinity, high temperature, extreme pH). The best way to find  
123 stable biocatalysts is to prospect microorganisms from extreme ecosystem capable of  
124 synthesizing stable catalysts.

### 125 **3. Various groups of biocatalysts for contaminants degradation**

126 Degradation of pollutants with the assistance of microorganisms is a sluggish method,  
127 which in actual reality reduces the viability of bioremediation (Ghosh et al., 2017; Varjani  
128 and Upasani, 2019). Microbial enzymes isolated from the cells were used for bioremediation  
129 during the last few decades along with use of microbes to address the above shortcomings

130 (Camenzuli et al., 2013; Nigam, 2013; Sonune and Garode, 2018). Biocatalysts are complex  
131 groups of macromolecules that induce a variety of biochemical reactions affecting the  
132 pathways to degrade the pollutants (Kalogerakis et al., 2017). The various groups of  
133 biocatalysts based on their activities concerning pollutant degradation have been illustrated  
134 by fig.1.

135 “[Insert Figure 1]”

136 Bioremediation based on complete and partially pure enzymes may not rely on the  
137 proliferation of a single micro-organism in a contaminated ecosystem but the catalytic action  
138 of the microbial produced naturally enzyme. Bioremediation can be accomplished in  
139 nutrient-poor soil by using a refined enzyme. The use of biocatalytic biotransformation that is  
140 harmless to the atmosphere does not contain harmful side products formed by microbial  
141 biotransformation (Gianfreda and Bollag, 2002; Ruggaber and Talley, 2006). As industrial  
142 scale biocatalysts production is carried out specifically by submerged fermentation, most  
143 studies have concentrated on construction/application of this type of bioreactors. However,  
144 production of biocatalysts through solid state fermentation process possesses higher yields  
145 and less expensive (Dhakar et al. 2014). Different agro-industrial waste products have been  
146 utilized for production of biocatalysts for bioremediation (Fatma et al. 2010; Debnath et al.  
147 2020). In many cases, production of these biocatalysts can be enhanced by immobilizing the  
148 source organisms (Lin et al. 2008; Dong-chul et al. 2019; Bera et al. 2020). It is necessary to  
149 invent biocatalysts that are resistant to adverse conditions like alkaline or acidic pH, high  
150 temperature, high salt concentration owing to its applications in various industries. The  
151 biocatalysts used in hydrocarbon degradation have been reported to be produced by



152 extremophilic microorganisms (Patricia et al. 2020). Wentzel et al. (2018), have investigated  
153 production of ligninolytic enzymes, lipases, and protease from filamentous fungi and yeasts.  
154 Microorganisms such as fungi, microalgae and bacteria produce multicopper oxidase enzyme  
155 (laccase) which has versatile applications in various industries. Recombinant protein  
156 expressions have been used to increase productivity in shorter durations. Horseradish  
157 peroxidase (HRP C1A) was isolated from Horseradish plant and transferred to *E. coli* BL21  
158 through rDNA technology which could produce more quantity of HRP C1A and could  
159 degrade phenolic compounds. Similarly, carboxylesterase was isolated from human liver and  
160 was inserted to *E.coli* could degrade pesticides, chlorine, carbamates compounds etc ( Gupta  
161 et al. 2017; Gundinger, et al. 2017)

### 162 3.1. Oxidoreductases

163 Different groups of microorganisms and higher plants generate and secrete oxidoreductases  
164 to remove substances through oxidative coupling including the oxidation of compounds  
165 through moving electrons from reductants to oxidants resulting in the release of CO<sub>2</sub> and  
166 chloride ions. As a result of pollutant depletion Oxidoreductases energy or heat is produced,  
167 and for biochemical activities, microorganisms used it (Medina et al., 2017). Several  
168 pollutants such as 2,4,6-trinitrotoluene (TNT), chlorophenol, phenol, polychlorinated  
169 biphenyls (PCBs), nitroaromatic compounds, dyes (bromophenol blue, malachite green) were  
170 degraded using oxidoreductases. For example, Gram-positive bacteria *Bacillus safensis* CFA-  
171 06 produces oxidoreductase and it degrades the petroleum substances.

172 Different kinds of phenolic compounds are created by lignin degradation in nature binding  
173 with other compounds polymerization and co-polymerization has been transformed into

174 another form by oxidoreductases (Husain, 2006). The textile industry releases the color  
175 compounds into the environment which is degraded by different enzymes such as laccases  
176 and peroxidases (Jang et al., 2009). Annibale et al. (2004), have reported that *Panus tigrinus*,  
177 white-rot fungi secreted an extracellular oxidoreductase (lignin peroxidase, Mn-dependent  
178 peroxidase, and laccase) that have removed the color, phenols, and organic discharged from  
179 olive-mill wastewater.

180 A lot of microbial species synthesizes oxidoreductase enzymes leading to redox reactions for  
181 the removal of radioactive metals. The plant that belongs to the *Gramineae*, *Fabaceae*, and  
182 *Solanaceae* families and secretes enzymes extracellularly for soil pollutant degradation such  
183 as hydrocarbon-containing petroleum hydrocarbon and chlorinated compounds (Park et al.  
184 2006; Edwin-Wosu et al. 2016).

### 185 3.1.1 Oxygenases

186 Oxygenases are the key catalyst for the degradation of aromatic compounds, catalyzing the  
187 ring's cleavage in aromatic compounds. Based on the number of oxygen molecules involved  
188 in the cleavage, oxygenases enzyme have divided into two subclasses: monooxygenase and  
189 dioxygenase. Monooxygenase has been catalyzing the addition of one oxygen atom  
190 molecule. Dioxygenase has catalyzed the addition of two oxygen atom molecules.  
191 Microorganisms like *Pseudomonas* sp. able to degrade the pesticides excreting Glyphosate  
192 oxidase (GOX). It can also catalyze chlorinated compounds of a wide variety, herbicides, and  
193 various groups of pesticides (Tangahu et al., 2011).

#### 194 3.1.1.1. Monooxygenases and Dioxygenases

195 Through introducing one molecule of oxygen, monooxygenases catalyze the breakdown  
196 of aromatic substances and enhance their reaction and solubility. Monooxygenases have been  
197 reported to be active in desulphurization, denitrification, and dehalogenation (Arora et al.,  
198 2010). Based on their cofactor used, monooxygenases are categorized into two categories:  
199 P450 mono-oxygenases group and flavin-dependent mono-oxygenases (Gaur et al., 2018).  
200 P450 monooxygenase is a heme-containing enzyme, found both in prokaryotes and  
201 eukaryotes *Bacillus megaterium* BM3 can degrade fatty acid and aromatic compounds  
202 excreting a P450 mono-oxygenase enzyme (Gustafsson et al. 2004). Methane  
203 monooxygenase metabolizes halides including aliphatic substances, heavy metals, and  
204 aromatic hydrocarbons. As reported by Singh and Singh (2017) about the enzyme methane  
205 monooxygenase that comes in different forms. It may either occur in the cytoplasmic  
206 membrane or the cytoplasm. Monooxygenase like tetracenomycin F1 and quinol mono-  
207 oxygenase function without any cofactors isolated from *Streptomyces glauscens* bacterium  
208 and *E. Coli.*, respectively (Arora et al., 2010). Whole White-Rot Fungus Genome Sampling,  
209 *P. Chrysosporium* identified 150 genes in 16 gene clusters clustered within existing 12  
210 cytochrome P450 (CYP) families and 11 fungal CYP clans and one single P450 reductase  
211 portion in the fungus (Tuomela and Hatakka, 2011).

212 Dioxygenases catalyze the oxidation of the aromatic compounds by inserting two  
213 molecules of oxygen. Aromatic dioxygenases can be categorized according to their  
214 mechanism of action into (1) aromatic ring cleavage dioxygenases and (2) aromatic ring  
215 hydroxylation dioxygenases able to degrade the different chemicals by adding two molecules  
216 of oxygen into the ring and split the compound aromatic rings respectively (Ozer et al.,  
217 2019). *Pseudomonas putida* F1 produces Toluene dioxygenase which catalyzes toluene

218 degradation. It acts for several contaminants such as aromatic and aliphatic substances as  
219 dioxygenase (Muthukamalam et al., 2017). In the soil bacteria, catechol dioxygenases  
220 catalyze the conversion of aromatic precursors into aliphatic substances (Ali et al., 2017). A  
221 significant number of aromatic compounds from different chemical, medicinal, and dye  
222 factories are released into the environment. Dioxygenase breaks down the 1, 2-position of the  
223 aromatic ring to integrate two oxygen molecules into the substrate (Guzik et al., 2014).  
224 Figure 2 illustrate probable pathway of oxidoreductase (monooxygenase & dioxygenase) for  
225 the transformation of pollutants.

226 “[Insert Figure 2]”

### 227 3.1.2. Laccases

228 Oxidases and Laccases that produce copper, catalyzing the oxidation of a broad variety of  
229 aromatic substances and phenol groups found in the environment (Mai et al., 2000). By  
230 oxidizing the bonds, Laccase even decolorized azo dyes and transformed them into less toxic  
231 compounds found in the ecosystem (Legerska et al., 2016). Laccase produced by *Trametes*  
232 *hispidia* fungus could decolorize various pollutants. *Trametes versicolor* is also a good source  
233 of laccase and it was immobilized on porous glass beads. This immobilized enzyme could  
234 degrade a wide variety of toxins, such as heterocyclic aromatic compounds, phenolic  
235 compounds, and aromatic compounds containing amines. Laccase produced by *R. Practicola*  
236 is capable of degrading and bio transforming phenolic compounds (Dodor et al., 2004;  
237 Strong & Claus, 2011). Illustration of Laccase Mediator System (LMS) and its role to  
238 detoxify organic pollutants has been shown in figure 3.

239 “[Insert Figure 3]”

### 240 3.1.3. Peroxidases

241 Peroxidases, produced by bacteria, fungi, plants, and animals are widespread. Phenolic  
242 radicals that are formed by oxidizing phenolic compounds, which quickly become less  
243 soluble (Bansal and Kanwar,2013). Peroxidases are further split into three classes: Class-1  
244 intracellular enzyme including yeast-generated cytochrome-c peroxidase, ascorbate  
245 peroxidase (APX) formed by certain plant organisms, and bacterial catalase peroxidase.  
246 Class-2 includes lignin peroxidase (LiP) and manganese peroxidase (MnP) secreted fungal  
247 enzyme. Though Class-3 produces secreted peroxides from horseradish products, such as  
248 horseradish peroxidases (HRP) (Koua et al., 2009).

#### 249 3.1.3.1. Lignin peroxidases (LiPs) and Manganese peroxidases (MnPs)

250 Lignin peroxidases belong to monomeric proteins that are secreted by fungi like *Trametes*  
251 *versicolor* and *Phanerochaete chrysosporium*. It catalyzes toxic pollutant oxidation in the  
252 presence of hydrogen peroxide and alcohol-veratryl as co-substrate and mediator respectively  
253 (Xu et al., 2014; Abdel et al., 2013). Lignin peroxidases demonstrate excellent use in  
254 wastewater treatment and bioremediation. Degradation of lignin by bacterial peroxidases is  
255 much more selective in terms of specificity and thermostability associated with fungal  
256 peroxidases (Tuomela and Hatakka, 2011; Behbahani et al., 2016).

257 Manganese peroxidases are known as indirectly acting to degrade lignin and xenobiotic  
258 substances are extracellular enzymes produced by fungi. This enzyme catalyzes the  
259 degradation of some phenolic groups, aromatic compounds, and coloring agents (Balaji et al.,

260 2019). This enzyme has a great potential to remove excess different forms of colorants such  
261 as anthraquinone, triphenylmethane, and azo dye. Zhanga et al. (2016), had identified and  
262 purified manganese peroxide from *Tremetes* sp. 48424. In *Peniophora incarnata* KUC8836  
263 a gene (pimp1) responsible for the synthesis of manganese-dependent peroxidase was  
264 identified. Further, this gene has been expressed in the *Saccharomyces cerevisiae* fungi to  
265 estimate its potential to remove anthracene (Lee et al., 2016).

### 266 3.2. Hydrolases

267 Hydrolases are widely used for the bioremediation of the insecticides. These enzymes  
268 specifically break large peptide bonds, carbon-halide bonds, esters, etc. It can degrade  
269 carbazyme into 2-aminobenzimidazole. Microbe-secreted extracellular hydrolases facilitate  
270 the degradation of organic polymers which can move through cell pores (Babita et al., 2018).  
271 It is very efficient to bioremediate organophosphate and oil spills by using a hydrolytic  
272 enzyme.

#### 273 3.2.1. Lipases

274 Lipases are used to perform inter-esterification, esterification, hydrolysis, and alcoholics  
275 reactions (Prasad and Manjunath, 2011). They are widespread in existence, catalyzing the  
276 degradation of triacylglycerols into glycerol and free fatty acids (Shukla and Gupta, 2007).  
277 Due to lipase activity the amount of hydrocarbon in the polluted soil has been reduced  
278 (Ghafil et al., 2016). Verma et al. (2012), had optimized the process for the bioremediation of  
279 crude oils with lipases extracted from *Pseudomonas aeruginosa* SL-72

#### 280 3.2.2. Cellulases

281 Cellulase formed by microorganisms can be associated with cell envelope. These are  
282 mainly degrading the cellulose. In the textile and detergent industries, cellulose microfibrils  
283 produced during processes and pollute the environment. *Bacillus* species contain other  
284 alkaline cellulases, and *Trichoderma* and *Humicola* fungi contain neutral and acidic  
285 cellulases (Behera et al., 2017). These cellulases were used in the paper and pulp industries  
286 for the removal of ink during paper recycling (Karigar and Rao, 2011). Recently, Imran et al.  
287 (2016) had characterized cellulase from *Humicola* species which can tolerate and work in  
288 adverse conditions like extreme pH and temperature. It can be used for hydrogen bond  
289 breakdown in detergents and washing powders industries. Aslam et al. (2019) had isolated  
290 *Bacillus Amyloliquefacience*-ASK11 that was a good source of cellulase isolated from  
291 industrial leather-tanning waste.

### 292 3.2.3. Carboxylesterases

293 Enzyme carboxylesterases have catalyzed the degradation of ester bonds of carbamates,  
294 organophosphates, and other chlorinated organic compounds (Cummins et al., 2007). Yin et  
295 al. (2016) isolated a strain of *Pseudomonas aeruginosa* PA1 can able to synthesize  
296 carboxylesterases. This could absorb and degrade the mercury at the infected site.  
297 Carboxylesterases have hydrolyzed their ester bond using the prevalent path for the depletion  
298 of all types of pyrethroid insecticides. In a study, the active site of the carboxylesterases  
299 isolated has been modified for pyrethroid hydrolysis by *in vitro* mutagenesis (Heidari et al.,  
300 2004).

### 301 3.2.4. Phosphotriesterases

302 Initially isolated from soil bacteria *Pseudomonas diminuta*, hydrolyze a broad range of  
303 organophosphate (Romeh and Hendawi, 2014). Some marine bacterial species, such as  
304 *Thalassospira tepidiphila*, *Phaeobacter* sp., *Ruegeria mobilis* can degrade the coastal oceanic  
305 phosphate trimester. A bacteria *Geobacillus stearothermophilus* having the potential to  
306 hydrolyze compounds containing both lactone and organophosphate. Thermostable  
307 phosphotriesterase extracted from *Geobacillus stearothermophilus* bacteria is highly which  
308 can tolerate 100°C (Moshe et al., 2018).

### 309 3.2.5. Haloalkane dehalogenases

310 Haloalkane dehalogenases are bacterial enzymes that use a hydrolytic mechanism to  
311 cleave the carbon – halogen bond of halogenated aliphatic compounds. Halogenated  
312 substances are formed anywhere in the soil as a consequence of both natural and man-made  
313 actions and can be poisonous, mutagenic (Kotik and Famerova, 2012; Koudelakova et al.,  
314 2013). Nagata et al. (2015), had identified haloalkane dehalogenase in bacterium  
315 *Xanthobacter autotrophicus* GJ10 having the ability to degrade 1, 2- dichloroethane.

### 316 3.2.6. Proteases

317 Proteases are found in all living forms as bacteria and fungi, plants, and animals (Kuddus  
318 and Ramteke 2012). Most of the marine microorganisms capable of producing protease  
319 (Sivaperumal et al. 2017). Kumar et al. (2014) had reported about the degradation of the  
320 diesel oil *in vitro* up to 54% by the proteases obtained from *Pseudomonas fluorescens*.  
321 Similarly, proteases from *Geotrichum candidum* and *Cladosporium cladosporioides* could  
322 decompose 55% of the nonionic ethoxylated surfactants (Jakovljević and Vrvic 2018).



#### 323 4. Identification of transformation pollutants and their eco-toxicological assessment

324 Enzymes require multiple pathways to remove various ecological toxins, leading to the  
325 production of various metabolic compounds and the final product during the biocatalytic  
326 cycle. Researchers and scientists focus mainly on the absence of parent molecules in most  
327 degradation experiments, rather than evaluating intermediate metabolites, transformation  
328 pathways, and determining the toxicity of transforming products (Jian et al., 2020). Phenolic  
329 substrates are converted catalytically to phenoxy radicals by using peroxidases catalysts in  
330 the presence of hydrogen peroxide. The phenoxy radicals produced in the catalytic process  
331 can be coupled with each other or with other reactive substances (Torres-Duarte et al. 2010).  
332 Peroxidases group of biocatalysts could transform pentachlorophenol in a multistep pathway  
333 with an oxidative dehalogenation process to produce tetrachloro-1,4-benzoquinone. Further,  
334 tetrachloro-1,4-benzoquinone is degraded through reductive dehalogenations process.  
335 Similarly, Chloroanilines (an intermediate used in the synthesis of dyestuffs, agricultural  
336 chemicals, and pharmaceuticals) can be transformed to chlorophenol by peroxidase  
337 transformation (Torres-Duarte et al. 2010). There are many studies with respect to PAH  
338 transformation process with the application of different biocatalysts like lignin peroxidase  
339 and manganese peroxidase, PAH are generally oxidized to quinones and hydroxylated  
340 derivatives. These oxidized products are found to be more biodegradable. The pesticides  
341 belong to organophosphorus group could be transformed in to oxon (P=O) derivative by  
342 chloroperoxidase. This enzyme has a capability to replace the sulfur atom by an oxygen  
343 atom, transforming the phosphorothioate group to an oxon derivative (Torres-Duarte et al.  
344 2010).

345 Various molecular methods have been used to detect the degradation of products through  
346 the catalytic reaction. These include mass spectrometry, liquid chromatography with tandem  
347 mass spectrometry gas chromatography-phase electrospray phase ionization mass  
348 spectrometry, and liquid chromatography-electrospray time-off light mass spectrometry  
349 (Alneyadi and Ashraf, 2016). Lonappan and groups (2016) had developed laser diode  
350 thermal desorption-mass spectroscopy detects the transition components. Biototoxicity assays  
351 were used to evaluate the degree of contamination in waters. A very well-known acute  
352 toxicity experiments were carried out using green algae species to categorical data on water  
353 quality levels (Cristovao et al., 2011). Cibacron Blue 3GA's toxicity and its by-products have  
354 been investigated using *Daphnia magna* as research organism. The inhibition of microalgal  
355 growth was investigated using *Chlorella vulgaris*. MALDI-ToF-MS has been used to  
356 confirm by-products of pollutant degradation (Bayramoglu et al. 2019).

357

358 A growth inhibition bioassay of *Scenedesmus obliquus* was tested with soybean  
359 peroxidase-treated triclosan solution and compared with the untreated triclosan to analyze the  
360 toxicity (Li et al., 2016). The application of triclosan to the formulations at a level of 10- $\mu$ M  
361 was found to fully suppress the *S. obliquus* growth, while soybean peroxidase treatment of  
362 triclosan solution is gradually growing its growth. In another analysis complete elimination  
363 of triclosan solution toxicity was achieved within 2 h of the reaction time (Muhammad et al.,  
364 2019). Microorganisms like *B. subtilis*, *E. coli*, and *B. megaterium* were used to analyze the  
365 toxicity profile of untreated and treated substances through growth inhibition assay. For this  
366 reason, after the exposure of bacterial species to the solution for a given time, the amount of

367 total viable cells is counted (Muhammad et al., 2019). The toxicity reduction potential of the  
368 MnP-Tween 80 was tested using bacterial growth inhibition experiments for *B. Subtilis*.  
369 Reports revealed that a 24-hour MnP-Tween 80 treatment resulted in a total loss of *B.*  
370 *Subtilis* growth inhibition by Miconazole. The same treatment could decrease by 78% growth  
371 inhibition of *Pseudokirchneriella subcapitata* by sertraline (Inoue et al . 2015).To determine  
372 their toxicity with *Raphanus sativus* plants, initial as well as lacquer-treated Bisphenol-A  
373 solutions were subjected to phytotoxicity analysis. The root length and germination of the  
374 seeds were recorded after 5 days of dark incubation (Lassouane et al., 2019).

375 The pollutants like Diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole were  
376 treated with laccase, subjected to its toxicity analysis by using bacterial luminescence method  
377 for *Photobacterium leiognathi*, and were found to be nontoxic in nature (Alharbi et al. 2019).  
378 Phytotoxicity assay was also executed by using *Lactuca sativa* seeds for sulfamethoxazole  
379 after treatment with laccase. Untreated sulfamethoxazole solution decreased the root length,  
380 the result was opposite in treated case (Al-Maqdi et al. 2018). Copete-Pertuz et al. (2018),  
381 have investigated toxicity for laccase treated oxacillin solution by MTT assay using human  
382 liver cells-hepatoma (HepG2). Other methods to analyze the toxicity of treated pollutants  
383 with biocatalysts include Yeast Estrogenic Screen (YES) assay, *Vibrio fischeri* luminescence  
384 reduction test, Microtox assay, Algal viability test using the fluorometric indicator etc. (Ji et  
385 al. 2017; Becker et al. 2016; Yousefi-Ahmadipour et al. 2016; Naghdi et al. 2018).

386

## 387 **5. Immobilized biocatalyst for contaminants remediation**

388 Immobilization of biocatalyst for remediation of pollutants has been reported for their speedy  
389 biodegradation. Applications of immobilized biocatalysts have been summarized in Table 1

390 “[Insert Table 1.]”

### 391 5.1 Immobilization with inorganic materials

392 Immobilized enzymes used through adsorption with a wide variety of different inorganic  
393 materials for the simultaneous removal of hazardous pollutants. Organic oxides, for example,  
394 alumina titanium, silica, and iron oxides have been applied to immobilize oxidoreductases  
395 along with selective sorption of contaminants, phenolic compounds, synthetic dyes, and  
396 antibiotics (Yu et al., 2015; Bilal et al. 2019<sup>b</sup>). Minerals and carbon-based materials possess  
397 good sorption properties and high stability. These were successfully applied by enzymatic  
398 oxidation and subsequent adsorption to remove pollutants in a study conducted by Ding et al.  
399 (2016).

400 Spherical alumina pellets and Al<sub>2</sub>O<sub>3</sub> pellets were used for the adsorption covalent binding  
401 of laccase enzyme respectively. It was found that the immobilized laccase could decolorize  
402 industrial effluents rich in Reactive Black 5. The interesting part of this study was that dye  
403 was adsorbed in the support materials and it could be degraded through laccase. In this  
404 process, 80 percent of the Reactive Black 5 was adsorbed and only 4 percent was degraded by  
405 laccase (Jakub et al., 2019). In contrast, through covalent bonding, only 10 percent were  
406 adsorbed to the pellets of alumina, and 90 percent of the Reactive Black 5 were biodegraded  
407 by laccase. The major problem was the saturation of active sites in case of a covalent  
408 immobilization process. This happens due to the multipoint attachment of the biocatalysts

409 with immobilized surfaces. The efficiency of the enzyme for the removal of dye was lost in  
410 this case (Osma et al., 2010).

411 The benefits of using inorganic materials for immobilization of the enzymes are having  
412 exceptional mechanical and increased stability. In some cases, a functional group like  
413 hydroxyl facilitates immobilization and adsorption of toxic contaminants using inorganic  
414 materials simultaneously. In these cases, enzyme elution from the support can be restricted  
415 owing to its multipoint attachments. However, covalent bonds formation cannot even be  
416 omitted due to the existence of several functional moieties, which further decreases enzyme  
417 leakage. Even successful sorption of both contaminants from the atmosphere and their  
418 bioconversion products is facilitated by the existence of many functional groups. Applications  
419 of inorganic oxides drawn the attention of the researchers for industrial applications. This is  
420 due to its porous structure, more surface area, exceptional stability, and established  
421 morphology. Additionally, applications of inorganic oxides for along with immobilization of  
422 the enzyme and adsorption of contaminants results in high efficiencies in elimination.

## 423 5.2 Immobilization with organic materials

424 Biopolymers and synthetic polymers, apart from many inorganic materials, are also  
425 applied for immobilizing the enzyme and sorption of a hazardous compound simultaneously.  
426 The existence of various chemical moieties (C=O, -NH<sub>2</sub>, -OH, and COOH) facilitate the  
427 efficient immobilization of enzymes and the sorption of contaminants. The presence of  
428 functional moieties and their natural origin exhibit very high peptide affinity concerning bind  
429 the enzyme (Bilal and Iqbal, 2019). Polymers like starch, agar, carrageenan being used for  
430 adsorption and biodegradation of toxic compounds simultaneously (Loffredo et al., 2014).

431 Apart from these, the chitosan is also most widely used as an organic polymer for  
432 immobilizing the enzyme. In a study, laccase was immobilized with chitosan and it was  
433 applied to decolorize the effluents having Sulfur Brown GD and Sulfur Blue 15. Here 70% of  
434 Sulfur Brown GD and 80% of Sulfur Blue 15 could be removed from the dye solution of 200  
435 mg / L at pH 6.5. The interesting part of this study, it was not effective if a mixture of these  
436 two dyes was treated with the same conditions (Nguyen et al., 2016). Chitosan film has been  
437 also used to immobilize mushroom tyrosinase to degrade phenol derivatives in wastewater  
438 and to absorb quinone derivatives produced after the oxidation (Yamada et al., 2005). The  
439 synthetic polymers like polyvinyl alcohol and polystyrene are also used for the simultaneous  
440 biodegradation and adsorption of harmful compounds apart from the natural organics. There  
441 were so many studies that were carried out with these synthetic polymers for the binding of  
442 laccases or tyrosinases enzymes to degrade phenolic derivatives (Zhang et al., 2014).  
443 In research, horseradish peroxidase was immobilized with polyacrylonitrile membranes and  
444 studied its potential for phenol degradation (Wang et al., 2016) Further, the crosslinking of  
445 horseradish peroxidase was done with glutaraldehyde to stop the elution of biomolecules  
446 from the matrix. The major problem with cross-linking was the decreasing number of  
447 chemical moieties capable of adsorbing phenol. Besides, covalent binding, adsorption, and  
448 even encapsulation using polymer supports will immobilize a wide variety of enzymes.

### 449 5.3 Immobilization with hybrid and composite materials

450 Hybrids materials can be synthesized by linking both organic materials and inorganic and  
451 organic precursors. These materials are having more affinity to the peptides present in the  
452 enzymes due to their biocompatibility. The two biopolymers namely chitosan and alginate  
453 were combined followed by the crosslinking of chitosan with glutaraldehyde having an

454 alginate-filled pore space, where *Agaricus bisporus* tyrosinase made immobilized (Ensuncho  
455 et al., 2005). The alginate beads produced possessed excellent mechanical properties. This  
456 was subjected to the enzymatic conversion to study the phenol removal from the wastewater  
457 and further sorption of quinone. About 90% of the phenol was extracted under ideal  
458 conditions after 4h of the operation. The synthetic polymers and biopolymers are very  
459 effective for pollutant adsorption and enzymatic biodegradation. As part of hybrid material,  
460 chitosan was linked to the Diaion WK10 and WK20 through the weakly acidic cation  
461 exchange resins. Tyrosinase was then covalently immobilized with this to remove  
462 alkylphenols from aqueous solutions (Jakub et al., 2019).

463 Zhang et al. (2020), have developed a smart microfluidic device to prepare horseradish  
464 peroxidase (HRP) and zwitterionic polymers [poly(carboxybetaine methacrylate)] in order to  
465 find a solution to enhance degradation process for bisphenol A. It was found that, this  
466 immobilized HRP could degrade 99.42% of bisphenol A in 20 minutes.

467 Polyacrylonitrile was combined montmorillonite to create nanofibers. This was enriched  
468 with graphene oxide to facilitate the electron transfer. The hybrid nanofibers could  
469 immobilize the *Trametes versicolor* that synthesize laccase and could extract catechol (Li et  
470 al. 2011; Wang et al., 2014). Graphene oxide was added to it to enhance the enzyme's  
471 catalytic properties. The less concentration of the immobilized enzyme and weak sorption  
472 capacity of hybrid material is the main drawback of the proposed hybrid system. Poly (D, L-  
473 lactide-co-glycolide) and multi-walled carbon nanotubes were used to fabricate hybrid fibers,  
474 that were used for the encapsulation of laccase. This system was executed to remove  
475 bisphenol A through biodegradation (Dai et al., 2016). The synthetic groups of polymers like  
476 poly(acrylic acid), poly(vinyl alcohol), and polyamine combine with inorganic precursors

477 (clays, iron, and silica) to produce a hybrid system and stable material for entrapment of the  
478 enzymes. This hybrid material-enzymes-system was capable of biodegradation and  
479 simultaneous adsorption of phenols (Xu et al., 2015). Similar way, a hybrid membrane system  
480 was synthesized out of chitosan and iron ions in order to degrade the color by immobilized  
481 laccase enzyme (Wen et al., 2015). Covalently immobilized enzyme (Laccase) with  
482 nanozeolite - carbon nanotube composites were synthesized and used by researchers for  
483 degradation of Direct Red 23. The activity of free laccase was found to be 60%, while the  
484 nanocomposite retained about 80% of its maximal activity after 8 days of incubation  
485 (Mahmoodi et al., 2020). Removal of the dyes (Brilliant Blue G, Procion Green H4G, and  
486 Crystal Violet) by using immobilized laccase within polypropylene chloride (PP) film and  
487 poly(glycidylmethacrylate) system was also reported (Yakup et al. 2017). Representation for  
488 immobilizing biocatalysts with polymeric materials has been illustrated by figure 4.

489  "[Insert Figure 4]"

## 490 **6. Research needs and future directions**

491 The lustiness of biological materials such as enzyme and microbes in the form of  
492 biosorption or biocatalytic material; under unique environmental conditions is highly  
493 desirable. Lustiness is commonly referred to as the durability and consistency of the  
494 substance against a variety of critical criteria applicable to diverse drainage and contaminated  
495 habitats. It is also essential to design well-controlled reactor units and treatment methods to  
496 mitigate the discharge of designed biological material into the surroundings. To this end,  
497 work should offer a technical foundation for risk reduction, although modern technological  
498 advancements are being processed in the production of the materials to deal with critical



499 environmental concerns. Finally, a key element to remember is cost, for the functional  
500 application of engineered catalysts and biosorption components. Immobilization of the  
501 desired protein onto a suitable matrix requires a pure protein that can be purified by  
502 extraction, identification, and purification steps which enhance the cost and time of a  
503 biological process.

504 Another essential step towards implementation is the analysis of mixture and matrix  
505 effects on biocatalytic processes, as wastewater represents a complex mixture of inorganic  
506 and organic substances. Because municipal wastewater is a dynamic mixture of various  
507 compounds, the use of enzyme combinations that function synergistically with specific  
508 selectivity should be a crucial issue in improving bio-catalytic treatment processes. The  
509 essential factor is the use of biocatalysts with optimal pH and temperature within the  
510 wastewater spectrum to maintain elevated stabilities and activities. In most experiments, a  
511 redox mediator was required to dramatically boost the efficiency of the transition (Ashe et al.  
512 2016), that create additional pollution. Thus, research to improve the efficiency and stability  
513 of biocatalysts in wastewater is essential to make it available that do not require a mediator.  
514 In already developed industrial wastewater treatment applications, the enzymes are isolated  
515 mainly through membranes. Biofilm formation is one of the drawbacks of membrane system.  
516 Another drawback is the secreted extracellular enzymes can potentially interact with the  
517 immobilized enzymes on the membrane, leading to a loss of enzyme activity.

## 518 **7. Conclusions**

519 New developments in genetic and macromolecule engineering are unveiling enormous  
520 potential to increase the implementation of biochemical functions at the molecular level and

521 establish novel approaches and emerging environmental management challenges. Recent  
522 developments allowed creation of materials with new capabilities, offering a safe and cost-  
523 efficient method for handling emerging problem of remediation of recalcitrant pollutants and  
524 extracting useful goods from the product. While modern work activities have made  
525 substantial and promising strides toward this goal, important areas remain to be studied in  
526 depth until advanced biological materials can be used in action to address environmental  
527 concerns.

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**Figure Captions:**

**Figure 1:** Classification of biocatalyst on the basis of their activity

**Figure 2:** Pathway to illustrate activity of oxidoreductase (monooxygenase and dioxygenase) in degradation of phenol and other aromatic pollutants

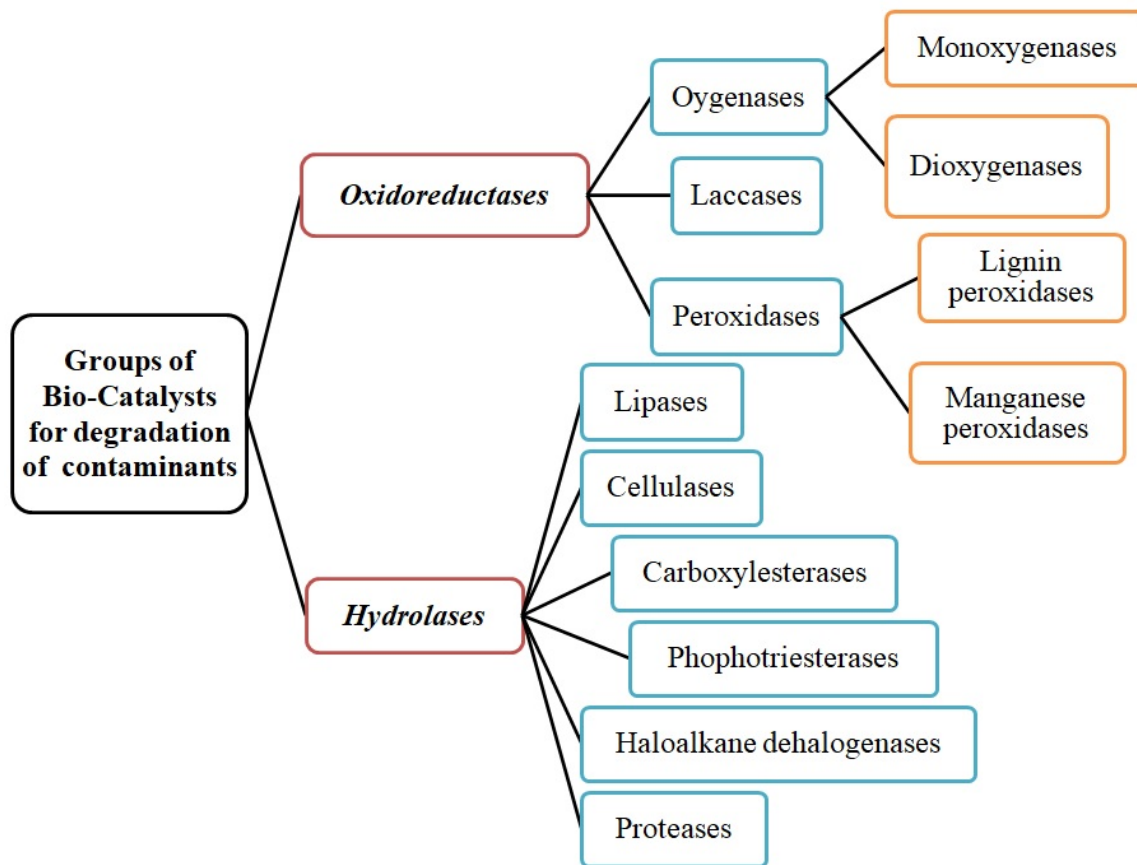
**Figure 3:** Illustration of Laccase Mediator System (LMS) and its possible role for bioremediation and detoxification of organic pollutants along with the active site of laccase to facilitate the catalytic cycle through electron flux

**Figure 4:** Schematic representation for immobilizing biocatalysts with polymeric materials and degradation of pollutants

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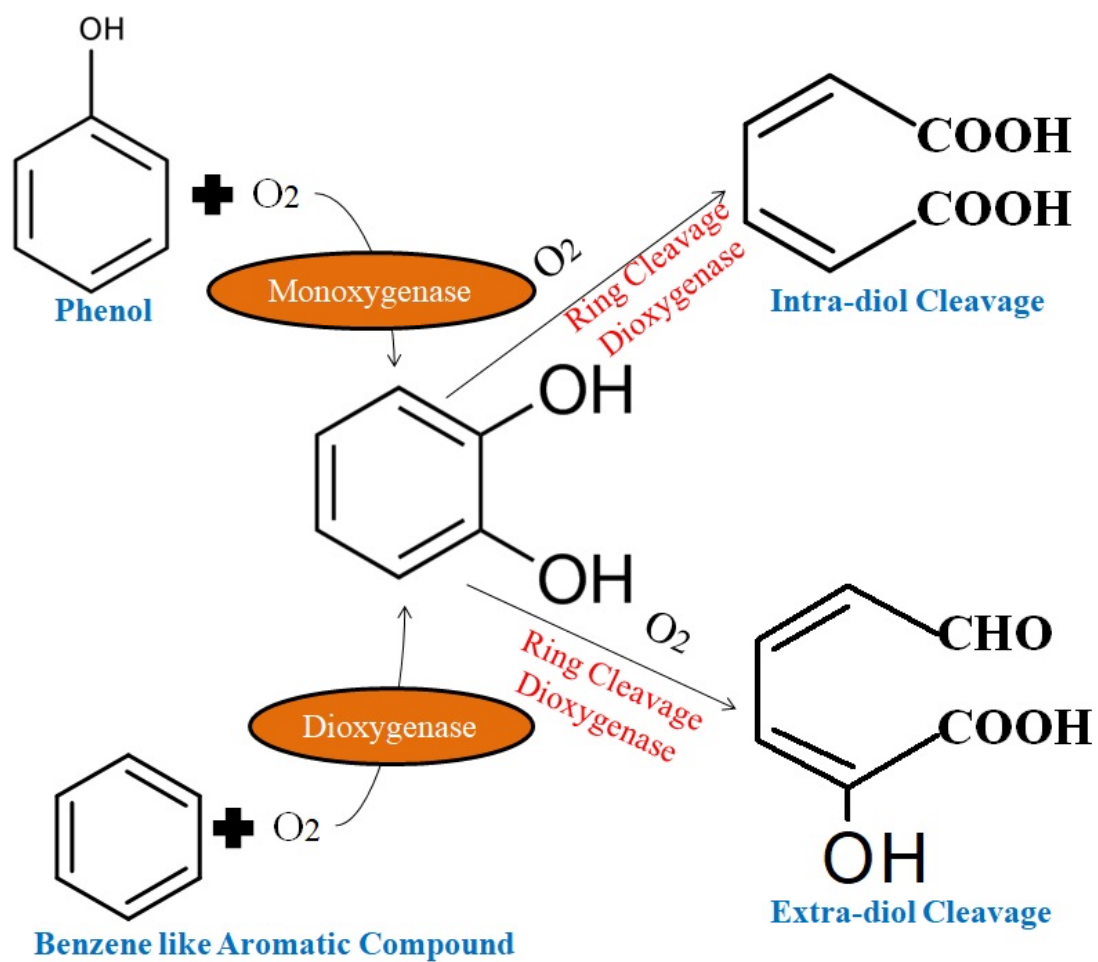
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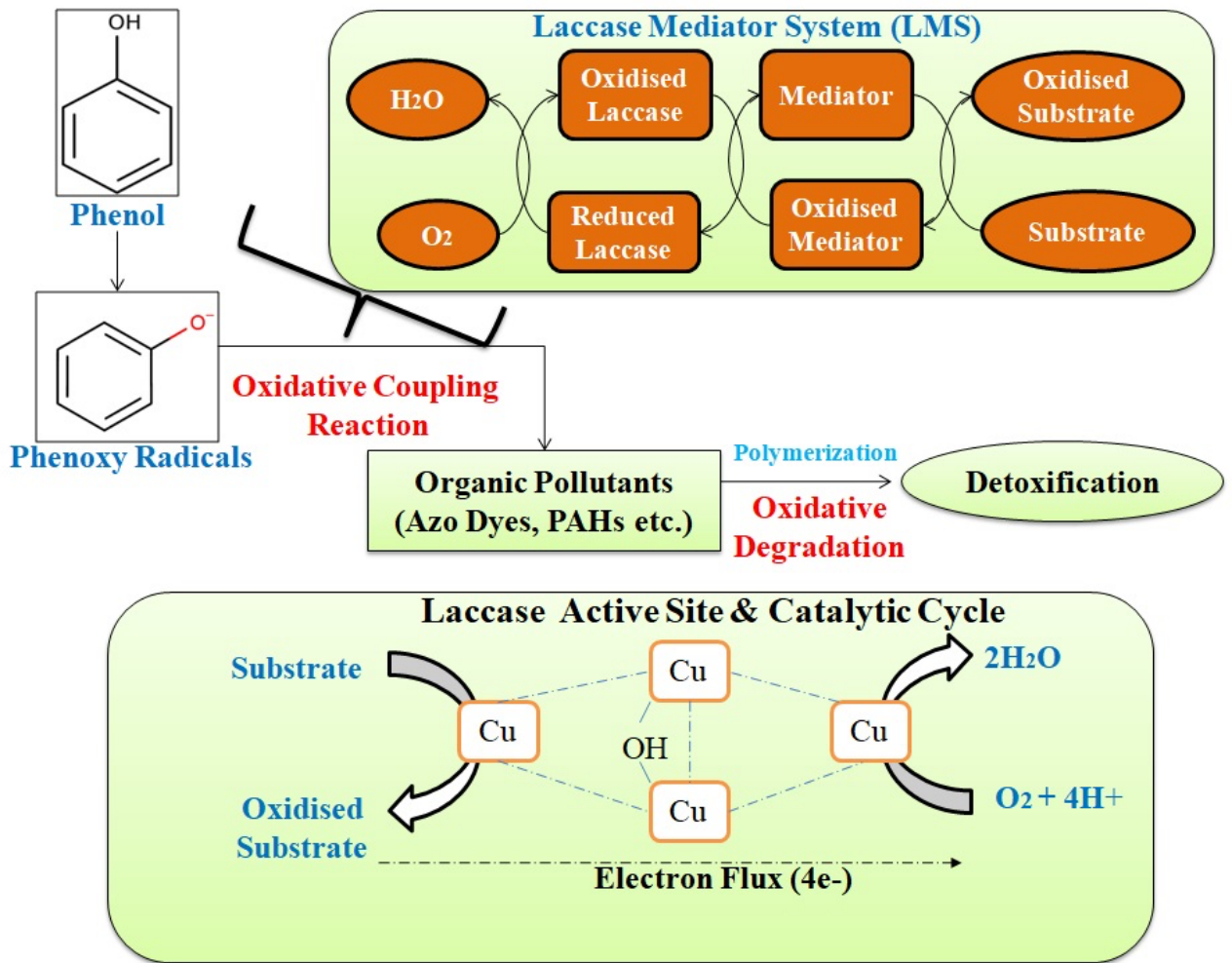
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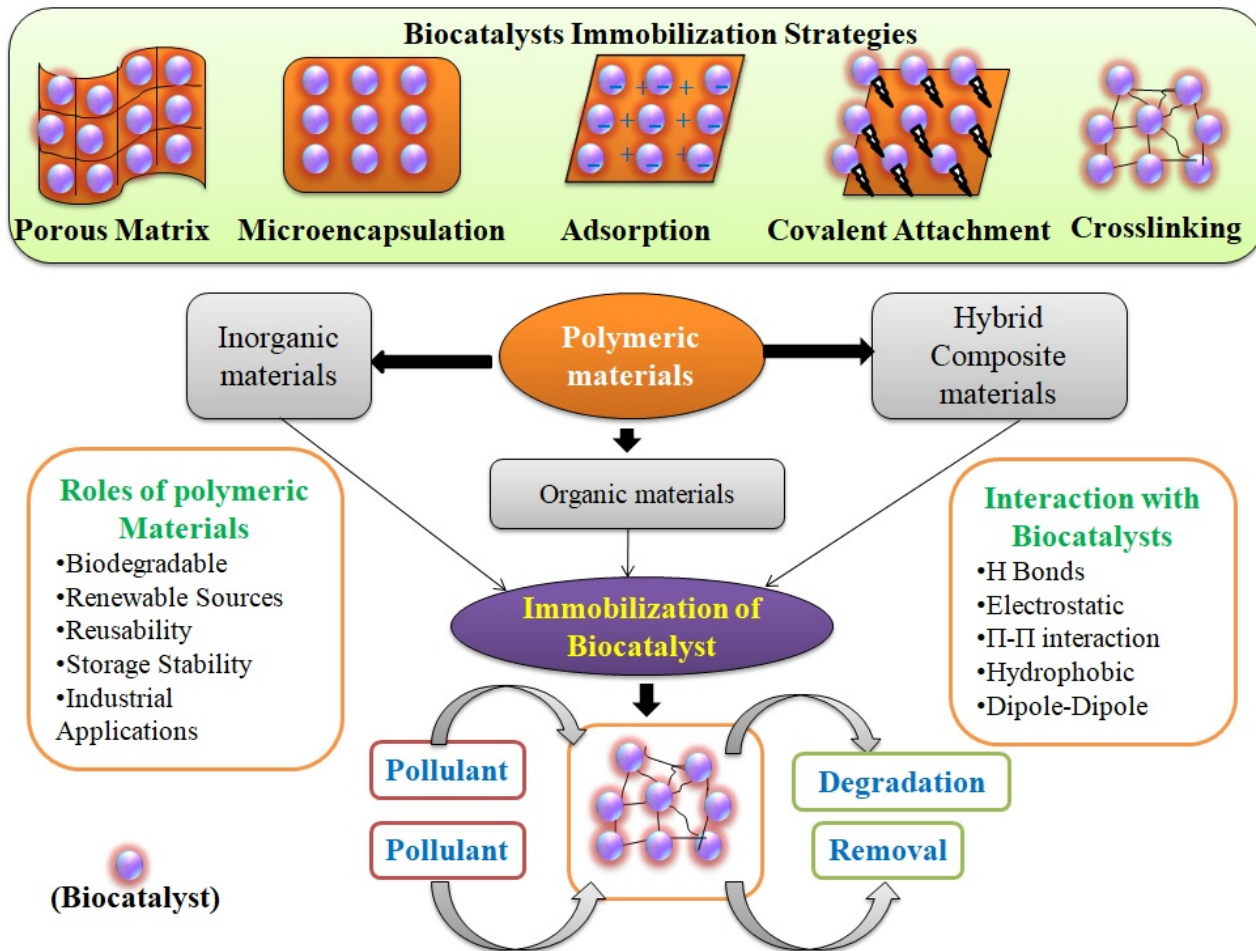
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**Figure 3:** Illustration of Laccase Mediator System (LMS) and its possible role for bioremediation and detoxification of organic pollutants along with the active site of laccase to facilitate the catalytic cycle through electron flux





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1084 **TABLE LEGENDS**

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1086 **Table 1:** Summary of immobilized biocatalysts for pollutants remediation

**Table 1: Summary of immobilized biocatalysts for pollutants remediation**

<b>Sr. No</b>	<b>Biocatalyst</b>	<b>Immobilization method</b>	<b>Target pollutant</b>	<b>Efficiency</b>	<b>Kinetics Parameters</b>	<b>Proposed methods of transformation</b>	<b>Reference</b>
<b>1</b>	Laccase	Crosslinking with Composite nanofibers	Triclosan	65% of Triclosan was removed in 2h	K for immobilized laccase = $1.17 \text{ h}^{-1}$ $t_{1/2}$ for immobilized laccase = 0.60 S First order reaction	Successive Oxidation, dechlorination, and oligomerization of Triclosan mediated through Cu-cluster in laccase	Xu et al. 2014
<b>2</b>	Laccase	Crosslinking with Magnetic mesoporous silica microbeads	Mefenamic acid, Indomethacin in Municipality Wastewater	N/A	$K_m = 64.3 \pm 6.7 \mu\text{M}$ $K_{cat} = 134.6 \pm 6.7 \text{ s}^{-1}$ $K_{cat}/K_m = 2.10 \pm 0.11 \text{ s}^{-1}\mu\text{M}^{-1}$ 2nd order kinetics	Resulting radicals from oxidation interact with nonphenolic pharmaceuticals	Arca-Ramos et al. 2016
<b>3</b>	Laccase	Covalent binding with Halloysite nanotubes ( $\text{Fe}_3\text{O}_4$ )	Sulfamethoxazole	60% of Sulfamethoxazole removed up to 7 <sup>th</sup>	$K_m$ for free laccase = $80 \mu\text{M}$ $K_m$ for immobilized laccase = $90 \mu\text{M}$	Oxidation with a redox mediator of laccase	Kadam et al. 2017

and functionalized with g-aminopropyltriethoxysilane)

cycle

$V_{max}$  for free laccase =  $45 \mu\text{M min}^{-1}$

$V_{max}$  for immobilized laccase =  $41 \mu\text{M min}^{-1}$

Lineweaver–Burk double reciprocal models

4	Laccase Crosslinking with magnetic nanoparticles	Acetaminophen, Diclofenac, Mefenamic acid, Atenolol, Epoxy carbamazepine, Fenofibrate, Diazepam, Trimethoprim, and Ketoprofen	Complete removal of acetaminophen, diclofenac, mefenamic acid, atenolol and epoxy carbamazepine and partial removal of fenofibrate, diazepam, trimethoprim, and ketoprofen was achieved within 12 h	$K_m$ for immobilized laccase = 0.39 mM $K_m$ for free laccase = 0.37 Mm	Direct oxidation of target pollutants by laccase	Kumar et al. 2016
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5	Laccase	Covalent binding with TiO <sub>2</sub> nanoparticles	Bisphenol A, Carbamazepine	90 % of the Bisphenol-A removed within 6 h and 40% of carbamazepine removed within 24 h t	For crude enzyme K <sub>m</sub> = 37.3 ± 2.5 μM K <sub>cat</sub> = 101.3 ± 11.8 μmol min <sup>-1</sup> mg <sup>-1</sup> For Immobilized crude enzyme K <sub>m</sub> = 42.9 ± 3.3 μM K <sub>cat</sub> = 75.5 ± 9.4 μmol min <sup>-1</sup> mg <sup>-1</sup>	Reaction of radicals with carbamazepine and Direct oxidation of bisphenol A	Ji et al. 2017
6	Laccase	Entrapment, covalent binding, crosslinking with Alginate, chitosan	Malachite green	90% of Malachite green decolorized in 3 h	N/A	Direct oxidation of tetracycline and pharmaceuticals by laccase	Yang et al. 2017
7	Horseradish peroxidase	Crosslinking with crosslinked enzyme aggregates	Methyl orange dye, Basic red 9, Indigo, Rhodamine B, and Rhodamine 6G	94.26% of methyl orange, 91.73% of Basic red 9, 84.35% of indigo, 81.47% of Rhodamine B, and 73.6% of	N/A	N/A	Bilal et al. 2017

8	Horseradish peroxidase	Crosslinking with chitosan–halloysite hybrid nanotubes	Tetramethyl benzidine	Rhodamine 6G was removed Crosslinked-Horseradish peroxidase showed 88 times faster catalytic activity with Tetramethyl benzidine than that of natural Horseradish peroxidase	V <sub>max</sub> of immobilized enzyme=260 mol min <sup>-1</sup> mg protein <sup>-1</sup> (88-folds higher than the free enzyme)	Direct oxidation by horseradish peroxidase in presence of H <sub>2</sub> O <sub>2</sub>	Kim et al. 2016
9	Horseradish peroxidase	Immobilized through adsorption with Magnetic nanoparticles (silica-coated), graphene oxide	Phenol	95% of phenol was removed from aqueous solution.	N/A	Direct oxidation by horseradish peroxidase in presence of H <sub>2</sub> O <sub>2</sub> . This phenomenon is probably due to different phenoxy	Chang et al. 2016

		(nanosheets), graphene oxide/Fe <sub>3</sub> O <sub>4</sub> , NH <sub>2</sub> -modified magnetic Fe <sub>3</sub> O <sub>4</sub> /SiO <sub>2</sub>				radicals produced by the enzyme	
10	Soybean peroxidase	Crosslinking with Silica-coated magnetic nanoparticles	Ferulic acid	99.67 ± 0.10% of ferulic was removed	Free enzyme Km=607.03 mM Vmax= 6.21 mM min <sup>-1</sup> Immobilized enzyme Km=21.55 mM Vmax= 0.654 mM min <sup>-1</sup>	Oxidation of phenolic compounds in presence of H <sub>2</sub> O <sub>2</sub>	Silva et al. 2016
11	Soybean peroxidase	Adsorption with Poly (styrene-co- maleic anhydride) (SMA) nanofiber	Diclofenac, Naproxen, Iopamidol, 2, 4- dichlorophenol, Imidacloprid, Bisphenol A	Complete removal of diclofenac and 2,4-dichloropheno, 90% removal of naproxen, 85% removal of imidacloprid and 70% removal of	Free enzyme Km (iopamidol) = 2.17 × 10 <sup>-4</sup> min <sup>-1</sup> Km (imidacloprid) = 2 × 10 <sup>-4</sup> min <sup>-1</sup> Km (bisphenol A) = 2 × 10 <sup>-4</sup> min <sup>-1</sup> Immobilized enzyme	Photocatalytic and enzymatic oxidation	Sarro et al. 2018

				iopamidol and bisphenol A was attained in 24h	$K_m$ (iopamidol) = $12 \times 10^{-4} \text{ min}^{-1}$ $K_m$ (imidacloprid) = $9.33 \times 10^{-4} \text{ min}^{-1}$ $K_m$ (bisphenol A) = $7.17 \times 10^{-4} \text{ min}^{-1}$		
12	Tyrosinase	Crosslinking with graphene oxide	Phenol, Bisphenol A	84.5% of phenol was removed after 2 h and 74.5 % of Bisphenol A was after 2 h	Free Tyrosinase $K_m=0.70 \text{ mM}$ $V_{max}= 4.43 \times 10^{-3} \text{ mM/s}$ Immobilized Tyrosinase $K_m=3.98 \text{ mM}$ $V_{max}= 0.9 \times 10^{-3} \text{ mM/s}$ Lineweaver–Burk double reciprocal models	Hydroxylation of monophenols to quinines	Liu et al. 2016
13	Organophosphorus hydrolase	Crosslinking with nonwoven fabrics	Methyl parathion	Complete removal of Methyl parathion was attained	Free enzyme $K_m= 331 \pm 2 \mu\text{M}$ Immobilized enzyme $K_m= 622 \pm 182 \mu\text{M}$	P-nitrophenol is produced with cleavage of P-O bond of methyl parathion	Gao et al. 2014



14	Organophosphorus hydrolase	Covalent binding with carbon nanotube paper	Methyl paraoxon	22% of paraoxon removed	Methyl paraoxon was removed	N/A	P-nitrophenol is produced with cleavage of P-O bond of methyl parathion	Mechrez et al. 2014
15	Lipase	Entrapment with Zeolite imidazolate framework-8	p-Nitrophenyl caprylate	N/A	N/A	N/A	Hydrolysis to produce p-nitrophenyl	He et al., 2014
16	Haloalkane dehalogenase and epoxide hydrolase	Encapsulation with PVA particles, lentikats	1,2,3-Trichloropropane	97% of Trichloropropane was converted to final product with 78% yield	1,2,3-Trichloropropane removed	The specific activities of immobilized Haloalkane dehalogenase and epoxide hydrolase were noted 29.5 and 6.5 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ respectively	Dehalogenation and hydrolysis process give glycerol	Dvorak et al. 2014
17	Laccase	Adsorption with magnetic silica microspheres	Phenolic contaminants	80% of phenolic contaminants were removed in 5 days	Phenolic contaminants were removed	For free enzyme $K_m = 1.0 \pm 0.12 \mu\text{M}$ $K_{cat} = 7.69 \pm 0.12 \text{ s}^{-1}$ $K_{cat}/K_m = 7.69 \pm 0.19 \text{ s}^{-1} \mu\text{M}^{-1}$	Free radical chain reactions break phenolic ring	Vishnu et al. 2017

For immobilized enzyme

$K_m = 2.0 \pm 0.19 \mu\text{M}$

$K_{cat} = 4.40 \pm 0.23 \text{ s}^{-1}$

$K_{cat}/K_m = 2.20 \pm 0.16 \text{ s}^{-1} \mu\text{M}^{-1}$

18	Formate dehydrogenase, peroxidase, and NADH oxidase	Adsorption with agarose beads	Phenol, Para-aminophenol, 2,4-dichlorophenol	95% of Phenol, 50% of Para-aminophenol, 70% of 2,4-Dichlorophenol, and 91% of Naphthol were removed	N/A	Formate dehydrogenase forms $\text{H}_2\text{O}_2$ through oxidation and NADH oxidase oxidize phenolic compounds	Rocha-Martin et al. 2014
19	Laccase	Covalently attached in Zeolite based nanoparticle and graphene oxide composites	Synthetic Dyes (Direct Red 23)	91% decolorization was found	N/A	Phenoxy radical formed with the oxidation of phenolic ring and followed by formation of azo linkage	Mahmoodi et al. 2020

20	Laccase	Cross linked with Sepharose-linked antibody support	Phenol red	Decolourization of phenol red dye obtained by immobilized and free laccase was 80% and 56%, respectively after 6 h of incubation	Free enzyme Km= 43.9 $\mu\text{M}$ Vmax= 4938 $\mu\text{M}\cdot\text{min}^{-1}$ Immobilized enzyme Km= 55.0 $\mu\text{M}$ Vmax= 408 $\mu\text{M}\cdot\text{min}^{-1}$	Phenolic ring structures has been breakdown with free radical reactions	Zofair et al. 2020
21	Polyphenol oxidase	Crosslinked with Chitosan/montmorillonite and chitosan-gold nanoparticles /montmorillonite composites	Phenol, 4-chlorophenol, 2,4-dichlorophenol	Phenol, 4-chlorophenol (4-CP) and 2, 4-dichlorophenol (2, 4-DCP) 89.2%, 95.2% and 93.8% at 480 min respectively	For phenol Km=376.8(mg/L) Vmax= 215.5 (mg/L/h) For 4-CP Km=365.7(mg/L) Vmax= 221.7(mg/L/h) For 2,4-DCP Km=370.2 (mg/L) Vmax= 217.9 (mg/L/h)	Catalyzes the oxidation of phenolic compounds into highly reactive quinones	Wang et al. 2020