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REVIEW ARTICLE

Laccase-assisted Bioremediation of Pesticides: Scope and Challenges

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Abstract: Laccase (Benzenediol: oxygen oxidoreductase; E.C.1.10.3.2), a multicopper oxidase that is a known lignin-degrading enzyme, can catalyse an ample array of substrates, from phenolic, non-phenolic compounds, aromatic amines, diamines, heterocyclic compounds to organic/inorganic metal compounds *etc.*, bestowed they have not too high redox potentials. Despite many laccase-producing organisms like bacteria, insects, plants, and animals, white rot filamentous fungi are the best producers of this enzyme. In the presence of laccase, pesticides (fungicides, herbicides, insecticides, *etc.*) of various chemical compositions (organophosphates, organochlorines, carbamates, pyrethrin & pyrethroids *etc.*) are oxidized into the water with collateral reduction of four electrons of molecular oxygen with various efficiencies. Bioremediation efficiency can be increased in the presence of various natural or synthetic mediators, *viz*. ABTS, violuric acid, 1- hydroxy benzotriazole, vanillin, syringaldehyde, PEG, *etc.* Immobilized laccase on various supporting materials increased the enzyme's stability, reliability, and reusability for continuous application, particularly for industrial processes. The present review discusses the structure, catalytic cycle, general mechanism of oxidation, and various scopes and challenges of pesticide degradation by this multifaceted biocatalyst which could lead to a green sustainable environment.

Keywords: Laccase, pesticide, bioremediation, mediators, immobilization, oxidation, redox potentials, green chemistry.

1. INTRODUCTION

Pesticides are either single or a mixture of substances frequently utilized in present agricultural practices to efficiently produce various foodstuffs for destroying, controlling or preventing any pest attack, including vectors of human diseases, animal diseases, and invasive animal or plant species. To feed more than 7.90 billion population with a limited area of agricultural land, the use of pesticides is of the utmost necessity. However, the indiscriminate use of pesticides compromises the existence of the present biome and our successors. Approximately 2 million tons of pesticides (herbicides, insecticides, fungicides, nematicides, *etc.*) are produced and consumed [1]. More than 95% of sprayed herbicides and 98% of sprayed insecticides end up in places where their intended targets are absent, including nontarget species in the water, soil, and air [2]. Pesticides have a high risk of polluting the environment, including rivers, marine waters, streams, ground water, *etc.*, because of their widespread and extensive usage [3], and several pesticides are bioaccumulated and biomagnified through our food chain [4–6]. Moreover, pesticide exposure is linked to several detrimental health impacts, including respiratory issues, skin damage, congenital disabilities, mental disorders, depression, miscarriages, cognitive defects, hematopoietic cancer, and even death [7–9]. Sharma *et al.* [10] reported the occurrence of 385 million cases of unpremeditated pesticide poisoning globally per year as per the database of WHO.

For the sake of ecological sustainability and survival, removing these hazardous substances from soil, wastewater, and our ecosystem is imperative. Physical or chemical approaches (flocculation, photocatalytic degradation, ozonation, coagulation, and Fenton oxidation) to degrade organic pollutants are expensive, time-consuming, primarily ineffectual, and can result in the production of more recalcitrant compounds [11, 12]. More recently, researchers have investigated that microorganisms play a significant role in degrading different organic contaminants and pesticides. Bioremediation involves microorganisms, *i.e.,* fungi, archaea, and bacteria (either naturally occurring or introduced) in the degradation of pollutants [13]. It is well known that many bacterial and fungal organisms degrade organic contaminants metabolically and concurrently [14]. Microbial communities, mainly filamentous fungi and bacteria, can interact chemically or physically with various pesticides, leading to degradation or structural alterations and transformation of pesticide molecules. The soil's most significant pesticide breakdown pathway is microbial metabolism [15]. Therefore, effective bioremediation strategies should be implemented to elimi-

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nate these massive loads of xenobiotic compounds from our environment. Now-a-day involvement of microbial enzymes in the degradation of pesticides has shown great potential. According to Bharadwaj *et al.* [16], enzymatic bioremediation is the efficient and safest way to convert hazardous toxic pesticidal compounds to nontoxic or less toxic simpler molecules.

Laccase (Benzenediol: oxygen oxidoreductase; E.C. 1.10.3.2), a lignin-degrading enzyme, belongs to the multicopper polyphenol oxidase family. Laccase was initially reported from the Japanese lacquer plant *Rhus vernicifera,* presently named *Toxicodendron verniciflum* [17–19]. It is reported from many fungi, plant, bacteria, archaea, and insects. Laccase enzyme is a proficient catalyst used in various fields such as the pulp and paper industry [20], food processing industry [21], pharmaceutical industry, textile industry [22, 23], *etc.*, and bioremediation, including waste water detoxification and as biosensors [24, 25]. Laccase degrades phenolic compounds with low-redox potential, such as chlorophenols, phenolic dyes, substituted phenols, sulfur phenols, aromatic amines, *etc.*, in the presence of oxygen because of their low redox potential [26–28]. Due to the catalytic oxidation of laccase with oxygen, it is called a "green catalyst." Laccases have unique qualities that make them desirable for chemical synthesis, including thermostability, high solution stability, tolerance towards mild reaction conditions, and selectivity for phenolic structure [29]. In the presence of a suitable natural/synthetic redox mediator, laccase can also oxidize a wide range of substances from non-phenolic compounds, polycyclic aromatic hydrocarbons (PAH), chlorinated aromatic compounds, endocrine disrupting chemicals, synthetic dyes to many pesticides [30].

Although laccases have many applications, free laccase is commercially not feasible due to low stability, poor reusability, and high costs. Immobilization of laccase is a powerful tool to make laccase more industrially potential. The selection of the matrix support material for immobilization is crucial, and it is based on the properties such as high porosity, high surface area, and the existence of functional groups. The present review discusses recent and comprehensive up-to-date information on this versatile enzyme and the mechanism of degradation of some representative pesticides in the presence /absence of different mediators in free and immobilized conditions.

2. SOURCE OF LACCASES

2.1. Bacteria

Laccase has been reported from several bacteria. First, bacterial laccase was reported in *Azospirillum lipoferum,* having a significant role in phenolic compounds oxidation, cell pigmentation [31] and electron transport [32]. Other laccase-producing bacteria are *Bacillus subtilis* [33]*, Bacillus sphaericus* [34]*, Streptomyces galbus* [35]*, S. lavendulae* [36], *S. griseus* [37], *Marinomonas mediterranea* [38], *Sinorhizobium meliloti* [39], *Anabaena azollae* [40]. *Aquifex aceolicus*, *Leptothrix discophora* SS1, *Xanthomonas campesteris* (copA), *E. coli*, *Alpha-proteobacterium* SD21, *Gama-proteobacterium* JB, *Bacillus halodurans*, *Pseudomonas aerophilum* (pae1888), *P. fluorescens* GB-1, *P. syringae pv tomato* (copA), *P. putida* GB1 (cumA), *Thermus thermophilus* HB27, *P. maltophila* and actinomycetes like *Streptomyces psammoticus* MTCC 7334, *S. antibioticus*, *S. cyaneus* [41]*, etc.*

2.2. Archaea

Laccase has also been reported from very few archaea. One archaeal laccase isolated from *Haloferax volcanii* [42] oxidizes phenolics. A multicopper oxidase (McoP) enzyme has been isolated from the hyperthermophilic archaeon *Pyrobaculum aerophilum*. And it shows nitrous oxide reductase activity. Laccase has also been reported from *Haloarcula marismortui* ATCC 43049, *Natronomonas pharaonic* DSM2160, *Candidatus Nitropumilus martimus* SCM1, *Halorubrum lacusprofundi* ATCC 49239 [43].

2.3. Algae

Tetracystis aeria is the first green algae reported to produce extracellular laccase [44]. Some other algal species produced laccase-like enzymes within which *Chlamydomonas moewusii* had true laccase [45]. Spirulina platensis CFTRI laccase was isolated and purified from the blue-green algae to achieve homogeneity [46].

2.4. Fungi

Fungal laccases are best known for their capability of breaking down lignocellulosic material. These laccases have a higher redox potential and act on PAHs, phenolic compounds, aromatic amines, synthetic dyes, drugs, and many other substrates. Members of the ascomycetes, deuteromycetes, and basidiomycetes are recognized as well-known laccase producers [18, 47]. Among ascomycetous members, *Neurospora crassa*, *Monocillium indicum*, *Aspergillus nidulans, Polyporus versicolor*, *etc.*, are the producers of laccase. *Theiophora terrestris*, *Schizophyllum commune, Coriolopsis fulvocinerea*, *Phanerochaete, chrysosporium, Phlebia radiata*, *Trametes versicolour*, *Pleurotus ostreatus*, *Trametes versicolor*, and *Cerrena unicolor, etc.* are the basidiomycetes producing laccase. Other wood-decaying fungi include *Trametes. Hirsute* (*Coriolus hirsutus*), *T. villosa*, *T. gallica*, *Lentinus tigrinus*, *T. ochracea*, *Coriolopsis polyzona, Pleurotus eryngii, Cerrena maxima*, *etc.* are laccase producers [48]. *Pestalotiopsis* sp. is a deuteromycetous laccase-producing fungus [49].

2.5. Plants

Hikorokuro Yoshida, in 1883 reported first plant laccase and then by Gabriel Bertrand from the sap of Japanese lacquer plants *Rhus vernicifera*and *R. succedanea*, respectively. Several studies on laccases isolated from *Pinus taeda* [50], *Acer pseudoplatanus* [51, 52], *Aesculus perviflora* [53], *Nicotiana tabacum* [54], *Populus euramericana* [55], *Salvia rosmarinus* [56], *Zinnia elegans* [57], *Camellia sinensis* [58], *Mangifera indica* [59]. Levine reported [60] that laccases and laccase-like polyphenol oxidases are produced by several vegetables and fruits like cabbages, beet, potato, turnips, pear, apples *etc.*

2.6. Animals

Laccase or laccase-like phenol oxidases were isolated from insects. They contained a long amino-terminal se-

quence composed of some conserved cysteine, a charged and aromatic amino acid residue. Laccase-like phenol oxidase has also been isolated from different insects like *Lucilia cuprina* [61], *Sarcophaga bullata* [62], *Manduca sexta* [63], *Drosophila melanogaster* [64], and *Acanthamoeba castellanii* [65]. Two isoforms of laccase have been reported from *Tribolium castaneum* [66, 67].

3. PESTICIDES

Pesticides have been categorised broadly based on their origin, composition, usage, toxicity, method of action, function, and the pest they destroy. (Fig. **1**). Pesticides are also divided into organic and inorganic according to their chemical composition. Organic pesticides also contain chlorine, fluorine, phosphorus, and sulfur. Contrarily, inorganic pesticides comprise metals, sulfur, and other elements. Depending on the elements and functional groups present in the chemical structure, pesticides may be again classified into sub-classes they are (a) organochlorines, *e.g.,* DDT, (b) organophosphates, *e.g.,* malathion (c) carbamates, *e.g.,* carbofuran (d) Pyrethrin and Pyrethroids, *e.g.,* permethrin and (5) other compounds like triazines, phenoxys *etc.* Pesticides belonging to the same class have a similar structure, poisoning symptoms, mode of action, and safety rules. However, every pesticide has its target site governed by the mode of action [68].

3.1. Organo-chlorines

Organochlorine pesticides (OCPs), a broad class of chemicals used to fight and remove weeds, insects, bacteria, and fungi, are made of carbon, hydrogen, and chlorine. (Fig. **2**). Organochlorines such as BHC and DDT and their analogous compounds like aldrin, endosulfan, chlordane, dieldrin, endrin, and heptachlor are used mainly as insecticides around the world. They are colourless, crystalline, and organic solvent or lipid-soluble compounds and tend to aggregate in food crops and environments [69, 70]. The most frequently used OCPs in public health, agriculture, and combat against tsetse flies, termites, and mosquitoes are DDTs and hexachlorocyclohexanes (HCH) [71, 72]. DDTs and HCHs are often considered characteristic compounds to estimate the ecological status of OCPs as they significantly impact the environment [73]. Organochlorine pesticides are regarded as the most hazardous among the several pesticides used. Exposure to OCPs may cause symptoms such as sore throat, headaches, itchy skin, rashes, blisters on the skin, vomiting, dizziness, nausea, blurred vision, and occasionally even blindness. OCPs have a prolonged life in the environment because they are difficult to decompose compared to other compounds. The higher strength of carbon-chlorine bonds cannot be decomposed by hydrolysis [74]. Due to their semi-volatile nature, OCPs in the environment can quickly spread across a vast region by wind and rainfall [75, 76]. OCPs are lipophilic and can remain hidden in fat cells for long periods. With the increasing existence and adverse effects, DDT was banned worldwide at Stockholm Convention in 2001.

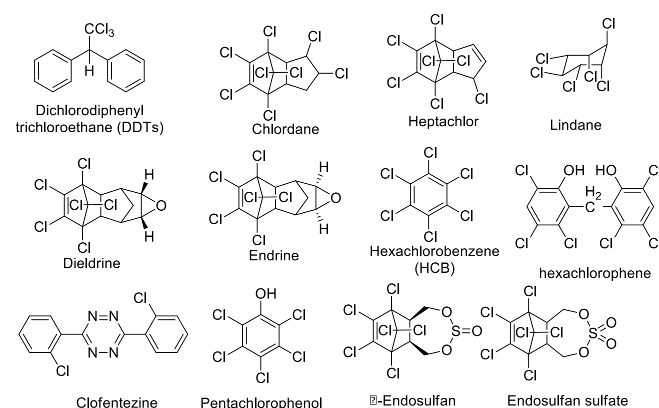


Fig. (2). Molecular structure of some organochlorine pesticides.

3.2. Organophosphates

Phosphoric acid ester groups containing pesticides are called organophosphorus pesticides (OPPs) and can increase agricultural crop yields in quality and quantity [77, 78]. Again, they were employed in the war as nerve agents like

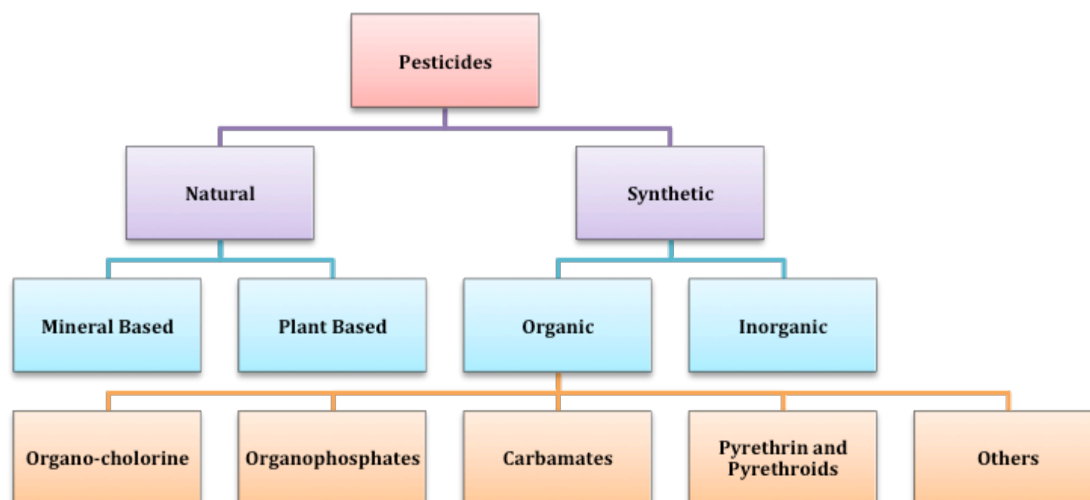


Fig. (1). Classification of pesticides according to the chemical structure. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

soman, sarin, tabun, and others. OCPs increased in the last decade because they effectively increase crop production. However, the widespread use of these pesticides poses a threat to aquatic life, the environment, and human health. Parathion, malathion, chlorpyrifos, fenitrothion, and phosphamidon are a few examples of OPP (Fig. **3**) applied in agricultural fields [78, 79]. These pesticides are acutely toxic to people when exposed at high concentrations as they irreversibly inhibit the acetylcholine esterase enzyme. This enzyme is crucial for nerve function in humans, insects, and many other species [78, 80]. Therefore, converting hazardous OPPs into safe chemicals is essential to reduce environmental pollution. OPPs are readily hydrolyzed by air, light, water, and soil, yet small quantities of these pesticides were found in food and drinking water [71].

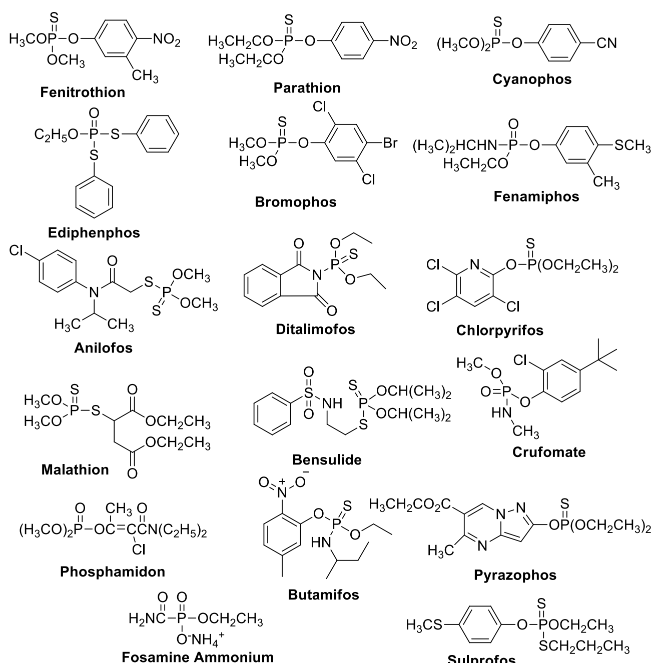


Fig. (3). Molecular structure of some organophosphate pesticides.

3.3. Carbamates

Carbamates are used as fungicides, insecticides, and herbicides obtained from the esterification of carbamic acid [81]. They inhibit acetyl-cholinesterase similarly to organophosphate pesticides, but their effect is reversible. It also shows the ability to combat a wide range of insects, and they are less toxic to human health [82, 83]. Because of their high solubility in water, carbamates are readily absorbed by plant roots and leaves [84]. They are used across the globe and are effective in preventing infectious diseases in many underdeveloped nations [85].

Carbamate pesticides (Fig. **4**) possess high acute toxicity. The carbamate insecticides contain an ester group of N-methyl carbamic acid(C_2H_5NO). This ester group takes a long period to break down in the soil [86]. The toxicities of carbamates are generally low, although there are some exceptions, like aldicarb. Carbamates are acetylcholinesterase (AChE) inhibitors, which are responsible for the accumulation of acetylcholine (Ach) in synapses and neuromuscular junctions [87]. ACh accumulates due to the inhibition of AChEs hydrolysis, causing various symptoms like excessive salivation, perspiration, and seizures [88]. Compared to organophosphorus, carbamate and insecticides are less harmful to human exposure [89]. Carbamates act like organophosphate pesticides by inhibiting Acetylcholine esterase, except that the action is reversible and the toxicity effect is shorter in duration.

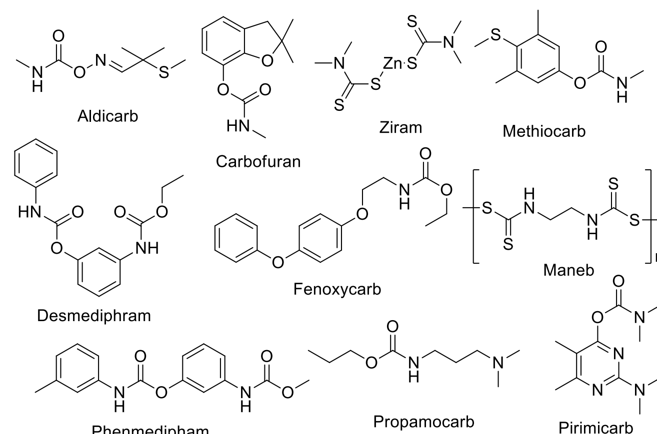


Fig. (4). Chemical structures of some carbamate pesticides.

3.4. Pyrethrin and Pyrethroids

Pyrethrins (Fig. **5**) are among the earliest known natural insecticides and were first utilised in the 1800s. [90]. Naturally occurring pyrethrins are composed of six organic compounds: pyrethrins I and II, jasmolin I and II, and cinerin I and II. Moreover, several synthetic derivatives are called pyrethroids, which have higher chemical stability than natural pyrethrins. Pyrethroids are again categorized as first and second-generation pyrethroids. The first-generation pyrethroids are esters of chrysanthemic acid and alcohol that contain a furan ring with terminal side chain moieties. "Second-generation pyrethrins" possess 3- phenoxybenzyl alcohol derivatives in the alcohol moiety, and some of the terminal side chain moieties have been changed to aromatic rings and dichlorovinyl or dibromovinyl replacement. The stability and insecticidal effectiveness of second-generation pyrethroids have improved due to the addition of the alpha-cyano group to the 3-phenoxybenzyl alcohol group.

Pyrethroids only exhibit high toxicity to various insects and low toxicity to birds and mammals and degrade rapidly. Pyrethroids act as an insecticide by delaying the closing of the inward sodium channel of the nerve membrane. Investigations revealed that pyrethroids might cause oxidative stress [91–95]. Traces of this is found in various organs, tissues, and cells, such as the liver [91, 93, 95], brain [91, 93, 94], kidney [91] and erythrocytes [96]. It is believed that the antioxidant enzyme like dismutase and catalase present in erythrocytes breaks down H_2O_2 and glutathione (GSH). Therefore, the increase in reduced glutathione (GSH) content in erythrocytes may probably be an initial adaptive response to increased oxidative stress in pyrethroid-intoxicated rats.

4. STRUCTURE OF LACCASE

Laccases are purified from a large number of fungal organisms. X-ray diffraction study of the crystal structure of the purified enzyme showed the 3D structures of laccases

[97]. Fungal laccases are monomeric glycoproteins with M.W. around 60-70 kDa, and it is also evident that the oligomeric forms of laccases also exist in nature. Laccases are glycoproteins, and glycosylation generally varies between 10% and 25% [98]. Carbohydrate molecules are attached to the polypeptide chain through *N*-linkages [99] and possess various roles like structural protection from proteolytic degradation [100] and enhanced thermostability [101].

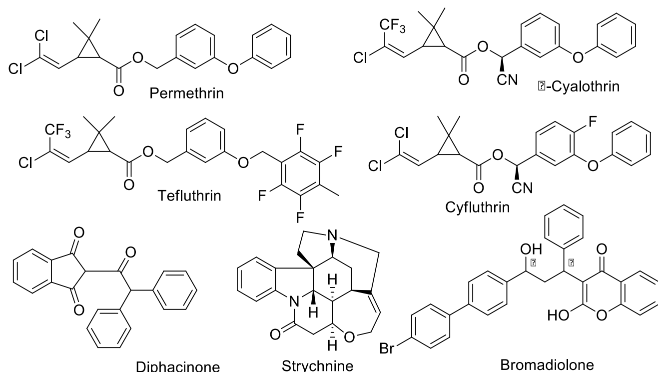


Fig. (5). Few examples and structures of Pyrethrin and pyrethroids.

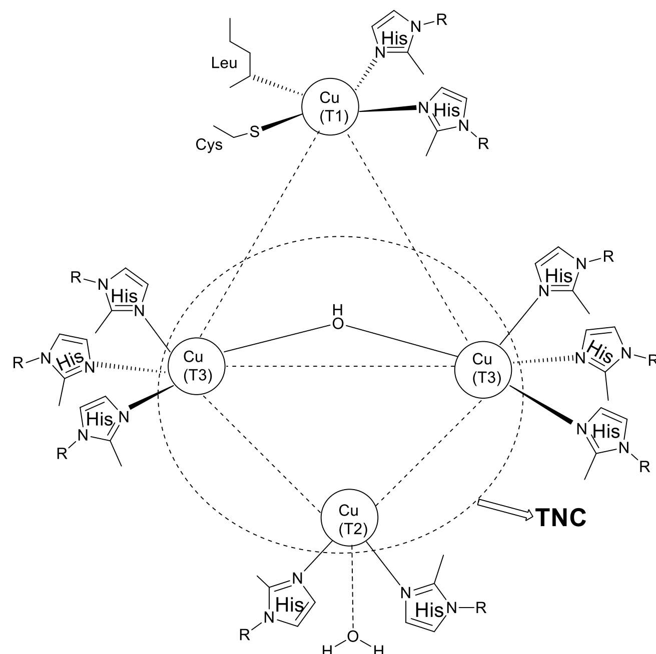


Fig. (6). Schematic representation of the active site of the structure of laccase enzyme [102].

Three cupredoxin-like domains with Greek key b-barrel topologies are consecutively assembled in fungal laccases. Laccase active sites typically contain four copper atoms, and they are of three types, viz. Type 1, Type 2 and Type 3 (Fig. **6**) [103] according to their EPR and other spectral characterisation [104, 105]. T1 Cu is positioned at domain 3, and the trinuclear Cu cluster (TNC) comprises type 2 and type 3 Cu, extending from domains 1 to 3 [106]. In the case of fungal laccases, T1, Cu has the planar trigonal geometry by coordinating with the S of Cys unit and Nd1 atoms of two His residues [106]. In most fungal laccases, the axial ligand is absent at the T1 Cu site. And the effect of this absence is minimized by a stronger Cu-S_{Cys} bond [107]. The geometric arrangement of the three Cu ions of TNC is triangular. Three His units are coordinated with each of the two T3 Cu ions and are bridged *via* hydroxide (OH), whereas the T2 Cu ion is coordinated to two His units by forming Cu-N bonds and one water molecule [108]. The Type-1 Cu shows a strong absorption band at 605–610 nm due to $S_{Cys}\rightarrow$Cu charge transfer and gives the characteristic blue colour in the oxidized state [107]. This blue colour makes it easy to differentiate 'true' laccase from 'yellow' or 'white' laccase. The characteristic shoulder peak of type 3 copper appeared at 330 nm, while type 2 copper does not show any peak in the absorption spectra. Type 1 and Type 2 copper are identified by hyperfine splitting in EPR spectra, while a pair of type 3 copper atoms do not show any signal due to strong anti-ferromagnetic coupling [109].

The hydrophobic residues, His, and acidic residues made up of Asp and Glu are primarily associated with the substrate binding site. Structural and mutagenic studies reveal that this amino acid part with an acidic group (Asp or Glu) has an important role in the oxidation of phenolic or amine substrates. It is proposed that the deprotonated carboxylate can form H-bonds with OH or NH_2 functional groups, resulting in deprotonation [110, 111]. Thus, the *p*H of the medium is essential as the enzyme must be present in the carboxylate form to perform its activity, and the *p*H required for this is around *p*H 5 or more. Nowadays, there are various computational methods to predict the potential mechanism of oxygen transfer to TNC, indicating the presence of a purposefully built channel [112].

5. REDOX ACTIVITY

For the catalytic oxidation process, laccase depends on three types of Cu discussed in the earlier section. The phenolic and other electron donor substrates are oxidized at the active site of the laccase by one electron-producing radical of the substrate and react non-enzymatically. The electron first interacted with type1 Cu and then transferred to the trinuclear Cu cluster (TNC) comprised of type 2 & 3 copper, where oxygen is reduced to water. Type 1 copper acts as the primary electron accepter, which extracts electrons from the substrate and transfers it to the trinuclear site, and the oxidized form of the enzyme is regenerated.

Type 1 copper atom of laccase has an interesting redox behaviour. Studies on fungal laccases have revealed that the higher the bond distance of the coordinate bond between ligands and T1 Cu, the higher the redox potential [108, 113]. Its potential redox value of laccase determines whether the substrate will oxidize or not. The efficacy of catalysis, however, depends on both the structure of the reducing substrate and the binding site of the laccase, in addition to the redox potential difference [114–116].In laccases with high E^0, the bond distance between T1 Cu and N_{His} is more. An H-bond with highly conserved Glu and Ser residues is assumed to be the main reason for this elongated bond distance. The coordinated His unit is located at the same helix as the Glu unit drew this His unit away from the T1 Cu. The increased Cu-N distance is thought to make the Cu ion more electron deficient because of less contribution of the lone pair of electrons from N-His that may destabilise higher oxidation states and increase E^0. The oxidation efficiency directly depends on

the difference in redox potential for the substrates having a redox potential higher than 700 mV [114, 117]. Based on the redox potential value, different types of laccases are generally classified into low, medium, and high redox potential enzymes [118]. Compared to laccases from bacterial and plant sources, fungal laccases often have a larger redox potential [119]. Moreover, the redox potential of type 2 or type 3 copper centres does not affect the catalytic activity by an appreciable amount. By the same logic, it may be explained that the T1 Cu possesses higher redox potential due to the absence of a fourth axial ligand. Site-directed mutagenesis of the axial ligand reveals that when this ligand is modified to a coordinating Met unit from a non-coordinating unit, viz. Leu or Phe, the redox potential of T1, Cu is significantly reduced [120, 121]. Results from QM/MM molecular dynamics simulations have further supported this theory [122]. The differences in redox potential and oxidation are also influenced by other aspects, such as dipole orientation, solvent accessibility, and hydrogen bonding.

6. CATALYTIC OXIDATION MECHANISM

Solomon *et al.* [123] established the mechanism of the catalytic cycle and the geometric and electrical structures of intermediates using a range of spectroscopic techniques (Fig. **7**). A completely reduced laccase (where all copper ions possess +1 oxidation state) reacts with dioxygen in two consecutive steps of two electrons. Dioxygen first binds to TNC, then one electron from T2 Cu ion and one from T3 Cu ion are quickly transferred to the oxygen molecule to produce peroxy intermediate (PI) [124]. The peroxide intermediate possibly contains the peroxide ion O_2^{2-} and One of the oxygen atoms were connected to the T2 and one T3 Cu, and the other oxygen atom was connected to the other T3 copper ion [125, 126]. This peroxide intermediate is reduced in a second two-electron step, thus oxidizing the type 2 and the type 1 centre to form the native intermediate (NI), which is actually an oxo or hydroxo bridged product between the T3 and T2 Cu ions of TNC. Conversion from the peroxide to the native intermediate, the peroxide bond cleaved in a way that includes proton and electron transfer, is not straightforward. This process has an activation energy of about ~9 kcal/mol [127], which is consistent with a mechanism involving the reduction of peroxide by one electron. A highly conserved carboxylate group unit (usually Asp) close to the T2 Cu ion may help to stabilise the PI and is essential for O-O bond breaking, according to experimental and theoretical studies [128]. If the reducing substrate is unavailable, the native intermediate (NI) is either progressively changed to the resting state, with one oxygen atom terminally bonded as H_2O to the T 2 copper or reduced by the substrate in the catalytic reaction. It is believed that the rate of this transformation is slower than the enzyme's turnover rate. This implies that the resting form is not involved in the catalytic cycle. In this way, O_2 is reduced to H_2O *via* two two-electron steps.

7. LACCASE MEDIATOR SYSTEM

Catalysis reactions of laccase are classified into two types: direct oxidation (without mediators) and indirect oxidation (*via* mediators). The direct oxidation changes the substrate to the corresponding radical due to the direct interaction with the copper cluster [130]. The scope of laccase-catalysed oxidation reactions can also be expanded to include atypical substrates, such as those with E^0 that is higher than the redox potential of T1 copper ion [48] through the application of the laccase mediator system (LMS). Usually, laccase E° at the T1 site ranges from +475 to +790 mV, but using LMS enables the oxidation of substrate molecules with E° higher than +1100 mV [131, 132]. Again, the mediator plays a role like a diffusible electron carrier, which enables the oxidation of biopolymers with high mol. wt. (lignin, starch, or cellulose). Therefore, the mediators help to overcome the steric hindrance preventing the direct interaction between an enzyme and a polymer. They basically act as "electron shuttles" between the target substrate and laccase. Here the oxidation reaction occurs in two steps: firstly, the enzyme catalyses the oxidation reaction of the mediator, and subsequently, this oxidized mediator oxidizes the substrate (Fig.8). Therefore, for the smooth reaction, the mediator must possess several characteristics, including (a) small molecular size, (b) being an ideal substrate for laccase in both its oxidised and reduced forms, (c) stable compound and does not inhibit the catalytic reaction, and (d) the reactions must be cyclic in nature [133].

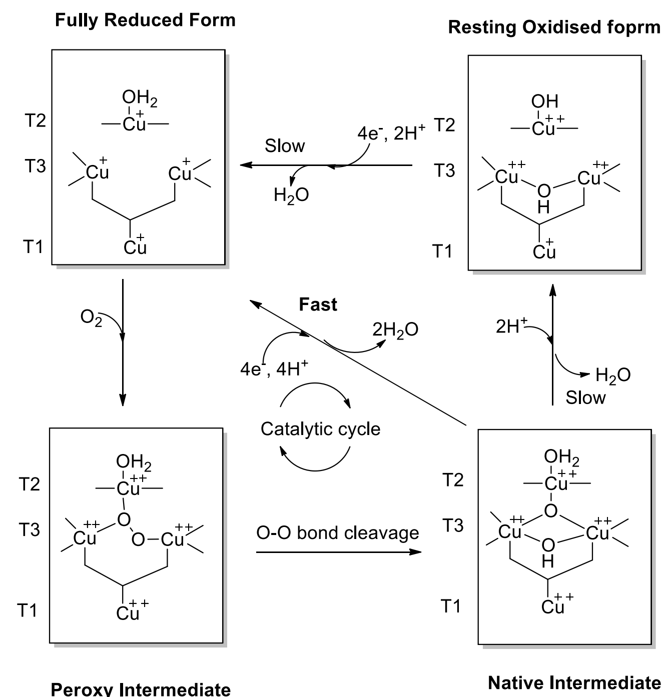


Fig. (7). Laccase catalytic cycle of oxidation [129].

Depending on the source, laccase mediators are natural and synthetic (Fig. **9**). Naturally occurring mediators resemble the structure of lignin-degradation compounds, such as *p*-coumaric acid, syringaldehyde (SA) and acetosyringone, etc [134]. These lignin derivatives are considered excellent mediators used in the LMS delignification of kraft pulp along with fungal laccases as delignifying enzymes [135]. Examples of synthetic mediators are 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) or ABTS, the *N*-hydroxy mediators, such as N-hydroxybenzotriazole (HBT), violuric acid (VLA), *N*-hydroxyphthalimide (HPI), *N*-acetyl-*N*-phenylhydroxylamine (NHA), and the radical TEMPO.

Although the most common redox mediators for laccase are ABTS and HBT, synthetic mediators are not financially viable and ultimately become toxic to the enzymes in the long run. Natural mediators have recently received great deal of attention due to their affordability and low toxicity. These naturally occurring mediators are mainly phenolic compounds that mediate the oxidation of lignin in white rot fungi.

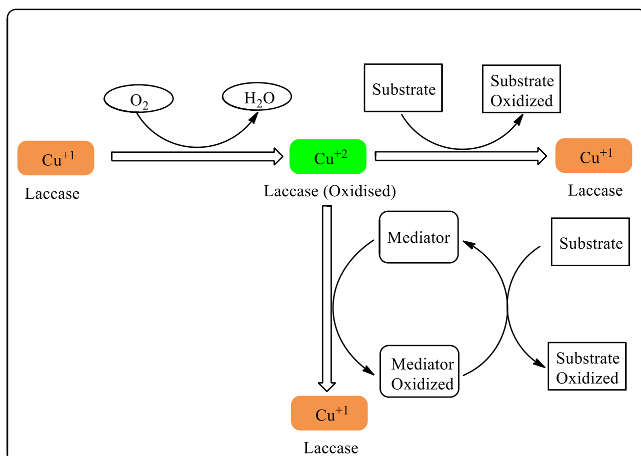


Fig. (8). Flow chart of oxidation reaction with/without laccase mediator system.

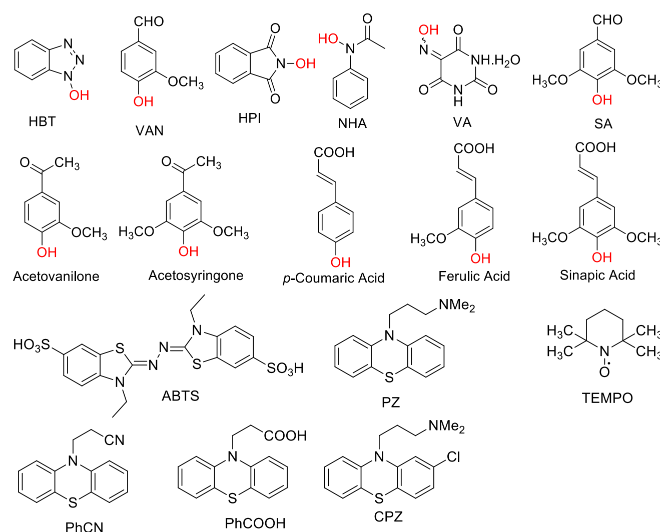
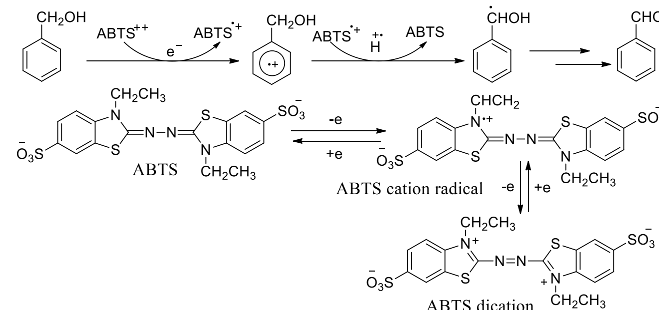


Fig. (9). Some common laccase mediators.

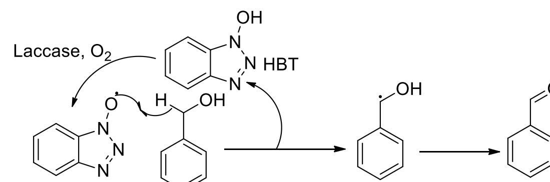
The mechanism of the reaction differs depending on the chemical structure of the mediator. Furthermore, different mediators on the same substrate may yield different products. This is mainly because substrate oxidation by laccase mediator reactions occur *via* different pathways. Because of their chemical structure and effective redox potential, mediator radicals preferentially perform a particular oxidation reaction. Various investigation suggests that, ABTS follows an "electron transfer" (ET) mechanism, and mediators like HBT that contain N-OH functionality follow a "hydrogen atom transfer" (HAT) route [136].

In contrast, TEMPO mediates *via* an "ionic" mechanism [137]. Natural mediators have some advantages over synthetic mediators, like less toxicity and less expensive, but they are generally less effective in the course of oxidation reaction [138]. The main reason behind this is that nitroxyl radicals formed by the oxidation of N-hydroxy substances are more stable than the respective phenoxy radicals generated by the oxidation of phenols, which means that nitroxyl radicals will remain for a prolonged duration and react more selectively.



Scheme 1. Electron transfer mechanism.

In the mechanism for ABTS type of mediator (Scheme **1**), only electron transfer causes the generation of free radicals, which results in the oxidation and reduction of the mediator. The first synthetic laccase mediator that performed oxidation of model non-phenolic lignin compounds was ABTS [139]. In the first step, the oxidation of ABTS produces the radical cation ($ABTS^{\cdot+}$); after that, this radical cation gradually oxidizes to the dication ($ABTS^{2+}$). The dication ($ABTS^{2+}$) oxidises the substrate. This reaction is driven by the irreversible two-electron oxidation process of the substrate, which regenerates radical cation ($ABTS^{\cdot+}$) [134].

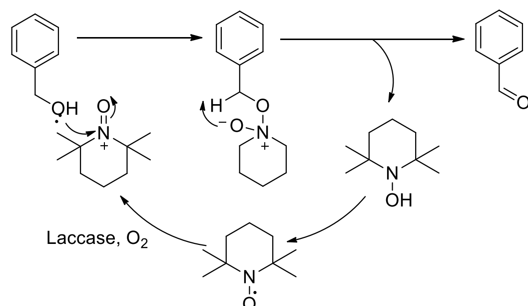


Scheme 2. Hydrogen atom transfer mechanism.

In HAT mechanism (Scheme **2**), the H^+ ion is also removed from the -OH group of the mediator besides an electron-producing free radical that performs main tasks of mediation [140]. The N-OH mediators are the most efficient for oxidation, such as HBT, HPI, VLA or NHA [141, 142]. Due to the enzymatic removal of an electron followed by the release of a proton, this type of mediator produces a highly reactive nitroxyl radical (N–O·) [143, 144]. The driving force behind this mechanism is the enthalpic balance between the dissociated bond (C-H) in the target substrate and the forming bond (NO-H) in the mediator [145, 146].

Commercially available TEMPO is stable, and it plays a significant role in the case of selective oxidation of 1° alcohol. The case of the nitroxyl radical TEMPO is quite different because it is oxidized by laccase, forming an oxoammonium ion (oxidation to N=O+ occurs at a redox potential that matches that of a high redox fungal laccase) that follows a non-radical-ionic-mechanism [143]. In the first step, TEMPO is oxidized to the corresponding oxoammonium ion (Scheme **3**). This cation can directly and selectively oxidise primary alcohols to yield the resulting aldehydes while simultaneous-

ly reducing the oxoammonium species to hydroxylamine [147]. Then hydroxylamine is converted to oxoammonium cation, and thus the catalytic cycle is completed. In such a system, oxygen can be used as a primary oxidant.



Scheme 3. Ionic mechanism.

8. IMMOBILIZATION OF THE ENZYME LACCASE

Enzyme immobilization is a significant development in industrial biotechnology. It helps to make the enzyme robust without compromising its biological activities. Immobilization of an enzyme is a common method to maintain the biological stability of the enzyme as well as to reuse the enzyme a number of times. It generates a continuous economic operation and recovery of the desired product with a great purity [148]. Hence, a useful tool to maintain the stability and functionality of an enzyme in a biotechnological industry. Immobilization is a technique of entrapment or imprisonment of the enzyme within a support matrix, an inert and insoluble material. The matrix allows the exchange of medium-containing substrate or inhibitor molecules concerning the enzyme. In addition, to being affordable, an ideal matrix must possess desirable characteristics like inertness, physical strength, constancy, renewability, enhanced specificity, decreased product inhibition, imprecise adsorption, and resistance to microbial contamination [149]. Several methods are used to immobilize the enzyme, like Adsorption, Entrapment, Encapsulation, Cross linking, and covalent bonding (Fig. **10**).

Adsorption results due to hydrophobic interactions and salt linkages. Hence, the technique uses water-insoluble carriers such as polysaccharide derivatives, synthetic polymers and glass [150–152]. Syringaldehyde (SA), a mediator, can enhance the laccase-catalyzed breakdown of nine pesticide residues, and biosorbents made from peanut shells and wheat straw can serve as supports for the immobilization of *Aspergillus* laccase [153]. The presence of side chain amino acids like arginine, aspartic acid, and histidine, as well as the degree of reactivity with various functional groups like imidazole, phenolic hydroxyl, *etc.*, cause **covalent bonding**. Multifunctional reagents such as glutaraldehyde, bisdiazobenzidine, and hexamethylenediisocyanate are utilised in the **cross-linking/covalent technique** [149]. **Encapsulation** is a process where the enzyme is surrounded by a capsule made up of semipermeable membrane like nylon or nitrocellulose. **Enzyme entrapment** is the confinement of enzymes within gels or fibers by covalent or non-covalent linkages. For the **entrapment** method, polymers including collagen, cellulose, and -carrageenan are used, but for the membrane confinement method, liposomes and microcapsules are created [154–157].

8.1. Support Matrix for Immobilization of the Enzyme Laccase

Immobilized enzyme becomes more vigorous and can resist various environmental alterations, allowing the enzyme's revival and reuse for multiple purposes. Different support systems for the immobilization of the enzyme can be employed (Table **1**).

Immobilization of the laccase enzyme solid matrix support could significantly increase the stability and enable its reuse resulting in cost reduction for the production of purified enzyme [158]. For example, laccase immobilized over rice straw biochar demonstrated improved stability after six usage cycles; immobilized laccase still retained 47% of its original activity [159]. Immobilising laccase on strong supports can increase its stability and reusability while boosting its activity. For the immobilization of laccase, various mate-

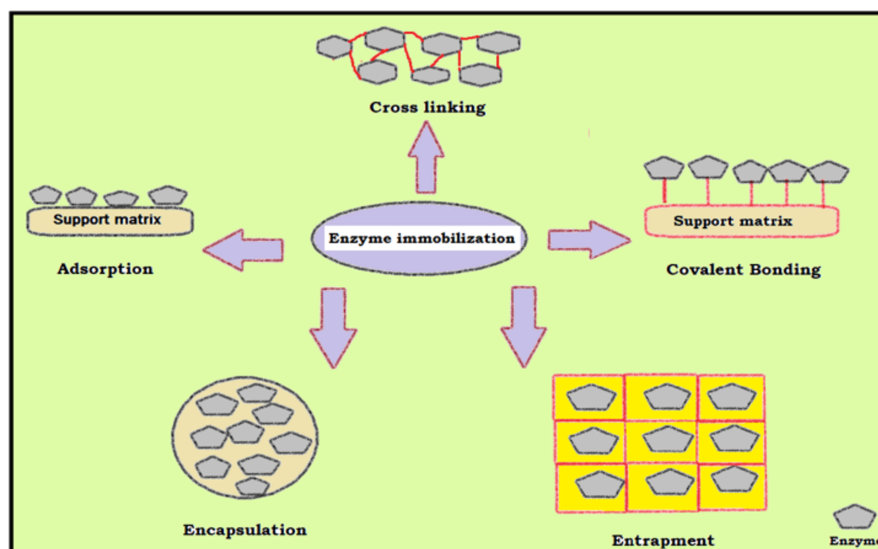


Fig. (10). Different methods employed for enzyme immobilization. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 1. Various types of support matrices were used for enzyme immobilization.

Sl. No.	Natural Polymers	Synthetic Polymers	Inorganic Materials
1.	Alginate	Amberlite	Silica glass
2.	Starch	Glutaraldehyde	Ceramics
3.	Cellulose	Polyethylene glycol	Zeolites
4.	Gelatin	Polyvinyl chloride	Activated carbon
5.	Sepharose	Polyaniline	Charcoal
6.	Chitosan & Chitin	Cyclodextrin	Celite

rials, including those with different chemical compositions like silica and inorganic materials [160], chitosan [24], and metal oxides [161], are utilised as solid supports. Among all diverse supports, carbon-based materials have been found as an ideal candidate for enzyme immobilization [161]. Carbon-based materials such as biochar, graphene, and activated carbons have been successfully used for enzyme immobilization [162]. Support materials are discovered to be a viable candidate for laccase immobilization when they have effective pore architectures, high surface area (up to 1000 $m^2 g^{-1}$), and a variety of functional groups on the surface [161]. Among all carbon-based materials, biochars are the most widely used because of their cost-effective and rational adsorption competence, making them a prospective candidate for laccase immobilization [163].

Lacasse produced by the fungus *Trametes versicolor* can be immobilized on the silica matrix (chemically adapted with imidazole groups and Amberlite IRA-400). Glass-ceramics can be chemically customized with the help of carbodiimide/glutaraldehyde and aminopropyltriethoxysilane/glutaraldehyde. The enzyme laccase is immobilized on various ceramic, pyrolytic graphite, and carbon fiber electrode substrates, where it can also function as a biosensor [164, 165].

8.2. Types of Laccase Immobilization

A large number of methods can be used to immobilize the enzyme laccase. The parameters for immobilization have to be optimized to enhance the enzyme activity. According to the literature, cross-linked enzyme aggregates (CLEAs) and magnetic CLEAs (M-CLEAs) can be created. The *p*H of the fermentation broth was brought down to 8.0 before immobilization. For CLEAs, laccase was precipitated with $(NH_4)_2SO_4$ at 25 °C for 2 hours, and 30 mM glutaraldehyde was used for crosslinking for 3 hours. For M-CLEAs, laccase was crosslinked for two hours with 40 mM glutaraldehyde before the addition of amino-functionalized magnetic nanoparticles (MNPs), which were supplemented at a laccase: MNPs ratio of 0.8:1 [166].

In the absence of redox mediators, *T. versicolor* laccase was immobilized using three different techniques: electropolymerized polyaniline (PANI) crosslinking, copper alginate bead entrapment, and Nafion micelle encapsulation [167].

For the entrapment method, at 60 °C, 2% alginate (w/v) was dissolved in distilled water. After fermentation, 2% alginate was added to the enzyme broth using a syringe, and the liquid was dropped over 2 % (w/v) $CaCl_2$ to create alginate beads. The alginate beads were then collected by filtration and repeatedly rinsed with distilled water in anticipation that no laccase activity was noticed in the water used for the washing process [166].

Cu-Alginate immobilization technique of Teerapatsakul *et al.* [168] can be used as a model. In 40 ml of water, 3% weight-volume sodium alginate was dissolved. Alginate solution and a 1U ml^{-1} laccase were combined, and the mixture was then slowly injected with a syringe into a 0.15 M copper sulphate cross-linker solution. The resulting beads were left undisturbed for 45 min before being rinsed with water and placed in an acetate buffer for incubation. The residual cross-linking solution and the laccase-immobilized Cu-Alg beads were then subjected to an enzyme assay to assess the immobilization yields [167].

Additionally, laccase can be covalently bound to chitosan. In order to create spherical beads, chitosan was dissolved in 1 % (v/v) acetic acid at a ratio of 3 % (w/v) and added dropwise over 2 M NaOH. Following filtering, the beads were collected, and distilled water was used to wash them repeatedly until the pH level was neutral. 1 gm of chitosan beads were added to 5 ml of 0.4 % glutaraldehyde, crosslinked for sixteen hours, and then rinsed with distilled water. Laccase was immobilized onto chitosan beads that had undergone glutaraldehyde crosslinking at a 1:1 (v/w) ratio for eight hours at room temperature [166]. Covalent bonding cross-linking is another prominent technique for the immobilization of laccase enzymes on various support surfaces [26]. In a different work, the immobilization parameters were optimized using covalent bonding to immobilize the laccase onto a customized polyacrylonitrile biochar composite nanofibrous membrane [169].

The immobilization of enzymes onto nanopolymers and nanoparticles has also generated considerable interest due to the tiny size of the enzyme carrier materials that increase the efficacy of the immobilized enzymes [170]. For usage in environmental applications, magnetic nanoparticles have the necessary magnetic characteristics. Magnetic iron oxide nanoparticles (MNPs) may be made cheaply, demonstrate physical and chemical stability, and are environmentally protected and biocompatible. MNPs are also employed in the

breakdown of pesticides and dyes, magnetic separation, and other processes [171, 172]. MNPs are among the viable materials for immobilizing enzymes among several types of nanoparticles because of their low toxicity and simplicity in recovery. Amin *et al.* (2018) and Dyal *et al.* (2003) demonstrated that the enzyme immobilized on MNPs displayed improved activity and stability [173, 174].

8.3. Comparison of Laccase Activity in Immobilization Methods

Laccase can be immobilized *via* entrapment, covalent binding, and cross-linking under optimized conditions. By using the same laccase, differences in activity recovery were then noted. An important element that affects the price of the immobilized enzyme is the activity recovery. Of all immobilized enzymes, laccase CLEAs recovered at a rate of around 68%, followed by laccase bound to chitosan at a rate of 50%, and laccase M-CLEAs at a rate of 47%, and entrapment at a rate of roughly 30%. (Table **2**). The following table summarises a comparison of free and immobilized laccase:Table **2**.

In one study [177], the enzyme laccase was immobilized on a variety of supports, including cotton, polyester, polypropylene, polystyrene, glass beads, and polyacrylamide gel. It was discovered that an enzyme's activity was decreased when it was immobilized compared to a free enzyme. The highest immobilized enzyme activity was discovered on glass beads (92%) out of various immobilization support systems. Polypropylene, polystyrene beads, polyester, and cotton threads were used separately and again in a modified adsorption method to increase the immobilization efficiency. The immobilization efficiency was observed to increase when they were coupled with glutaraldehyde, going from 61 to 86 % for polypropylene, 50 to 84% for cotton thread, and 52 to 75% for polyester threads, and 25 to 40% for polystyrene beads (Table **3**).

8.4. Thermostability of Immobilized Laccase

Due to the persistent conformational shift that occurs in the structure at high temperatures, enzymes become sensitive and may permanently lose their catalytic function. At 60 to 70 °C, the thermostability of free and immobilized laccases has been investigated. All laccases were thermally inactivated using a straight-forward first-order exponential decay model, which predicted a one-step transition from the dynamic to the denatured condition, either *via* the breaking of a crucial bond or the deformation of a crucial structure. At temperatures between 40 °C and 70 °C, it was shown that immobilized laccases had noticeably greater stability than free laccase; however, both types of laccases inactivated uniformly at 80 °C. At 70 °C, immobilized laccase was preserved with 21.9% relative enzyme activity, while free laccase was only 1.2% active. As a result, immobilized laccase performs better than free laccase in terms of thermostability, which may help to shield laccase from the hostile environment's high temperatures [178].

8.5. *p*H Stability of Immobilized Laccase

A study was done on how *p*H affected free and immobilized laccase [179]. Both free and immobilized laccase displayed their peak activity at *p*H 3. Both free and immobilized laccase's activity declined as pH was raised; however, at each *p*H level, immobilized laccase's activity was higher than free laccase.

The impact of *p*H on the free and immobilized laccase activity was also investigated at 30°C in the *p*H range of 2.0-7.0. The ideal *p*H for free laccase was found to be *p*H 4.0, which is in line with earlier research, whereas the ideal *p*H for immobilized laccase was changed to *p*H 5.0 [180].

8.6. Immobilized Lacasse in Biodegradation of Pesticides

As a whole ecosystem, soil, water, and air get contaminated due to industrialization and widespread pesticide use for increased agricultural output. Some of the pesticides that are known for their carcinogenic and mutagenic effects and are persistent in the environment include pentachlorophenol (PCP), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), isoproturon, dymron *etc.* For the treatment of agricultural soils, contaminated water, or polluted ecosystems, biodegradation of pesticides in the natural environment is crucial [181]. Although a number of pesticides are degraded in free condition (Table **4**) by the enzyme laccase, various procedures have been devised for the biological treatment of pesticides. Among them, cell and enzyme immobilization is a successful strategy that guarantees the biological processes (catalytic activity), which are maintained for an extended time. Two types of cell immobilization are reported. One is based on physical retention (*e.g.,* Entrapment), and the other is based on chemical bonding (*e.g.,* Biofilm formation) [182]. Numerous organic and inorganic supports, *viz*, starch, cellulose, dextran, alginate, agarose, chitin, keratin, and collagen, are used in cell immobilization techniques [183].

Aspergillus laccase was immobilized using biosorbents made from peanut shell and wheat straw, and the redox mediator syringaldehyde (SA) was used to speed up the laccase-catalyzed breakdown of nine pesticide residues, including isoproturon, atrazine, prometryn, mefenacet, penoxsulam, nitenpyram, prochloraz, and pyrazosulfuron. Pesticides were successfully eliminated from soil and water samples using immobilized laccase degradation on peanut shell or wheat straw supports [153]. The degradation of various pesticides by laccase in the absence and presence of mediators is summarised in Table **5**. In addition, laccase from different sources immobilized on various support matrices can also be utilised for the degradation of pesticides with varying efficiencies to make the environment clean (Table **6**).

9. LACCASE-ASSISTED DEGRADATION MECHANISM OF PESTICIDES

Yang *et al.* reported [204] degradation of methoxychlor by magnetic host/guest-type immobilized laccase (laccase-MHGI) (Scheme **4**). The process was started by reductive elimination of chlorine and subsequent removal of methoxy group by immobilized laccase along with ultrasmall superparamagnetic iron oxide nanoparticles (USPIO), which resulted in degraded intermediate **1** with m/z= 281. After being oxidised by laccase, the intermediate **1** continued to be degraded into compound **2** (m/z 205), was next undergone reductive cleavage by USPIO to produce anisole (m/z 108) and 1,1-dichloroethane (m/z 98) [205]. Then this undergoes hy-

Table 2. Comparison of free and immobilized laccases from some fungal species.

Sl. No.	Free/immobilized Laccase	Source of Laccase	Activity Recovery (%)	Refs.
1.	Free	*Cerrena* sp.	-	[166]
	CLEAs		68.1	
	M-CLEAs		46.8	
	Entrapped		29.8	
	Chitosan bound		50.1	
2.	Chitosan using glutaraldehyde	*Coriolus versicolor*	52.2	[175]
3.	Immobead-150 by covalent binding	*Pycnoporus sanguineus CS43*	97.1 ± 1.2	[176]
	Eupergit-C by covalent binding		83.2 ± 1.4	
	Entrapment in LentiKats		89 ± 1.1	

Table 3. Yield of laccase on various immobilization support systems.

Immobilization Support Systems	Source of Laccase	Relative Enzyme Activity (%) without Glutaraldehyde	Relative Enzyme Activity (%) with Glutaraldehyde
Control	Trametes versicolor	100	-
Glass beads		92	-
Polyacrylamide Gel		75	-
Polypropylene small pieces		61	86
Polystyrene beads		25	40
Polyester thread		52	75
Cotton thread		50	84

Table 4. Degradation of different pesticides by free laccase with or without mediators.

Pesticides	Mediator	Degradation (%)	Time (h)	Source of laccase	Refs.
Atrazine	HBT	90	192	*T. versicolor*	[7]
Bentazon	Vanillin	54	3	*T. versicolor*	[184]
Carbofuran	Vanillin	39	3	*T. versicolor*	[184]
Chlorpyrifos	Vanillin	100	48	*Pichia pastoris*	[185]
Chlorpyrifos	-	81	6	*Pseudomonas* sp.	[186]
Chlorpyrifos	Sinapic acid	83	6	*Pseudomonas* sp.	[186]
Chlorpyrifos	ABTS	70	48	*Lenzitesbetulinus*	[187]
Chlorpyrifos	-	29	15	*Tricholomagiganteum*	[188]
Chlorpyrifos	Vanillin	90	192	*T. versicolor*	[7]
Chlorothalonil	Acetosyringone	90	192	*T. versicolor*	[7]
Diuron	Vanillin	46	3	*T. versicolor*	[184]

(Table 4) contd….

Pesticides	Mediator	Degradation (%)	Time (h)	Source of laccase	Refs.
Dichlorophos	-	46	6	*Pseudomonas* sp.	[186]
Dichlorophos	Sinapic acid	46	6	*Pseudomonas* sp.	[186]
Dymron	ABTS	90	24	*Trametes*sp.	[189]
Glyphosate	ABTS	41	24	*T. versicolor*	[190]
Glyphosate	Mn^{2+}+Tween 80	63	24	*T. versicolor*	[190]
Glyphosate	ABTS+Mn^{2+}+Tween 80	90	24	*T. versicolor*	[190]
Isoproturon	Violuric acid	100	24	*T. versicolor*	[7]
Isoproturon	HBT	100	24	*T. versicolor*	[191]
Monocrotophos	-	75	6	*Psudomonas*sp.	[186]
Monocrotophos	Sinapic acid	79	6	*Psudomonas*sp.	[186]
Profenovos	-	82	6	*Psudomonas*sp.	[186]
Profenovos	Sinapic acid	82	6	*Psudomonas*sp.	[186]
Pyrimethanil	Violuric acid1	100	24	*T. versicolor*	[7]
Trilluralin	Guaiacol	100	24	-	[192]

Table 5. Degradation of different pesticides by Laccase.

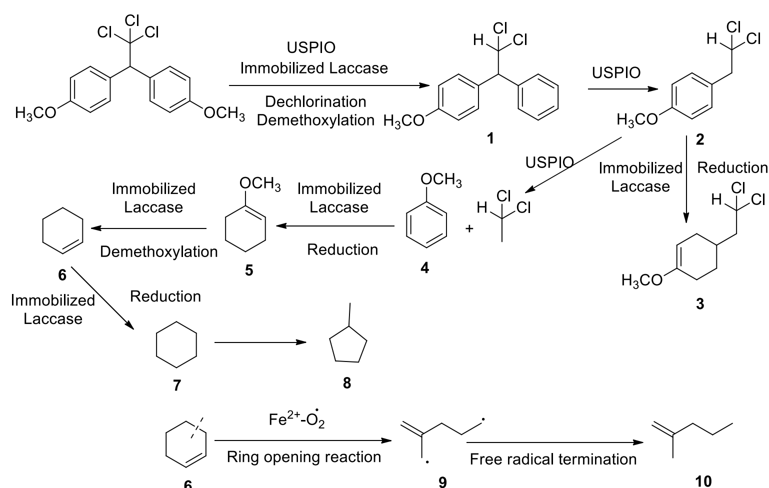
Pesticides	Source of Laccases	Mediator	Refs.
Atrazine	*Pleurotus ostreatus*	ABTS, VAN, SA, HBT, HPI, VA, TEMPO	[193]
Dymron	*Trametes* sp.	ABTS, HBT, NNDS and Violuric acid 4-Hydroxy benzoic acid (HBA), Methyl 4-hydroxybenzoate (MeHB)	[189]
Atrazine, Chlorpyrifos, Pyrimethanil, Isoproturon, Chlorothalonil	*T. versicolor*	ABTS, VAN, AS, HBT, VA, Guaiacol	[7]
Dichlorophos, Monocrotophos, Profenovos	*Pseudomonas sp.*	SA	[186]
Isoproturon	*T. versicolor*	ABTS, VAN, AS, HBT	[191]
Isoproturon, Atrazine	*O. sativa* laccases expressed in *Pichia pastoris*	-	[194]
Flubendiamide	*Neurospora sp.; Botryosphaeria rhodina*		[195]
Ametryn, Fenoprop, Atrazine, Propoxur, Pentachlorophenol	*M. thermophila* laccase expressed in *A. oryzae*	SA	[196]
Glyphosate	*T. versicolor*	ABTS	[190]
Isoxaflutole	*P. chrysosporium, T. versicolor,*	ABTS	[197]
Isoproturon, Atrazine, Prometryn, Mefenacet, Penoxsulam, Nitenpyram, Prochloraz, Pyrazosulfuron-ethyl and Bensulfuron-methyl	*Aspergillus* sp.	Syringaldehyde, Acetovanillone, Acetosyringone, Vanillin and *p*-Coumaric acid	[153]
3,5-Dichloroaniline derived from dicarboximide	*T. versicolor*	MnO_2, Catechol, Syringaldehyde, Syringic acid, Caffeic acid and Gallic acid	[198]
Simazine, Atrazine and Terbuthylazine, Propazine, Prometon, Atraton, Prometryn and Ametryn	*Pseudomonas stutzeri* sp. Y2	No mediator	[199]
Glyphosate, AMPA, Isoproturon	*T. versicolor*	MBTH, DMAB, ABTS	[200]

Table 6. Degradation of Pesticides with Immobilized Laccase.

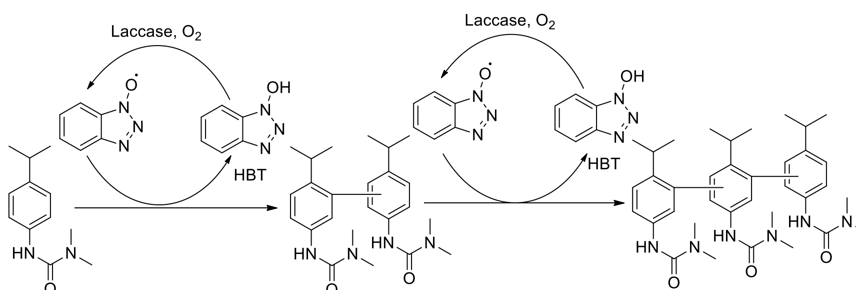
Pesticide	Source of Lacasse	Support Matrix	Efficiency/Maximum Laccase Activity Recovery	Refs.
Chlorpyrifos	*Bacillus* sp.	Iron magnetic nanoparticles (MNPs)	99%	[170]
Dichlorophen	fungal laccase	hybrid nanomaterial (laccase–MSU-F)	-	[201]
Isoproturon, Atrazine, Prometryn, Mefenacet, Penoxsulam, Nitenpyram, Prochloraz, Pyrazosulfuron-ethyl, Bensulfuron-methyl.	*Aspergillus* sp.	Biosorbents peanut shell wheat straw In the presence of redox mediator syringaldehyde (SA)	54.5% 65.9%	[153]
Carbofuran	Fungal lacasse	powdered active carbon (PAC)	86%	[68]
2,4-dichlorophenol (2,4-DCP)	*T. versicolor*	(i) soil & kaolinite	95%	[202]
		(ii) montmorillonite 1	69%	
		(iii) montmorillonite 2	42%	
		in the presence of aminopropyltriethoxysilane and glutaraldehyde		
Dichlorodiphenyltrichloroethane (DDT)	Polyporus sp.	immobilized reversed micelles laccase	Activity increased by 20%	[203]
Chlorpyrifos	*Bacillus* sp.	iron magnetic nanoparticles (MNPs)	99%	[170]
Dichlorophen		hybrid nanomaterial (laccase–MSU-F)		[201]
Isoproturon, Atrazine, Prometryn, Mefenacet, Penoxsulam, Nitenpyram, Prochloraz, Pyrazosulfuron-ethyl Bensulfuron-methyl.	*Aspergillus* sp.	Biosorbents peanut shell wheat straw In the presence of a redox mediator SA	54.5% 65.9%	[153]
Carbofuran	Fungal lacasse	powdered active carbon (PAC)	86%	[68]
Dichlorodiphenyltrichloroethane (DDT)	*Polyporus* sp.	immobilized reversed micelles laccase	Activity increased by 20%	[203]

drogenation to form 1-methoxy-cyclohexene (**5**) (m/z 112). Thus, demethoxylation of 1-methoxycyclohexene (**5**) could form cyclohexene (**6**) (m/z 82). Finally, the cyclohexene (**6**) molecule might be split into groups like vinyl, propane, butane, and pentene as shown in Scheme **4**. Secondly, with the help of laccase, the intermediate cyclohexene (**6**) and compound **2** underwent hydrogenation reaction and converted to cyclohexane (**7**) and compound **3**, respectively. Zhai *et al.* [206] reported that, during the GC/MS analytical process, compound **8** was the main pyrolysis fragment of cyclohexane. In the third step, cyclohexene was reacted with $O_2^{\cdot-}$, and produced radicals by the ring-opening process. The decomposition product, 2-methylpentene (**10**) (m/z 84) can be obtained by a radical termination reaction under the concurrent action of immobilised laccase and a reducing ferrous ion.

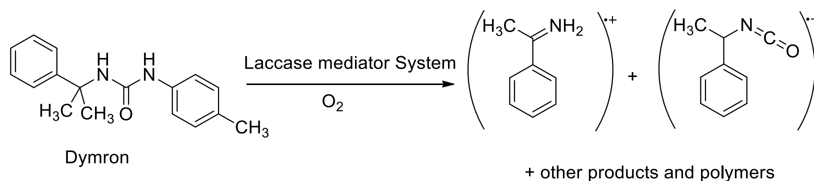
Zeng *et al.* reported [191] the degradation process of isoproturon by oligomerisation. During the study with laccase-HBT system, they found two additional peaks of the transformation products with m/z ratios of 619 and 413, corresponding to the trimer and dimer molecular weights of isoproturon in relation to the parent compound (m/z 206). Murugesan *et al.* previously reported [207] that oligomerization is a way to degrade the pollutant triclosan by utilising the laccase-HBT system. As a result, the radical-radical cou-



Scheme 4. Reaction mechanism of methoxychlor degradation by laccase-MHGI system [204].



Scheme 5. Laccase-HBT system catalyzed the degradation mechanism of isoproturon.

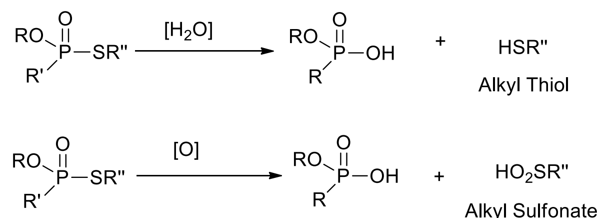


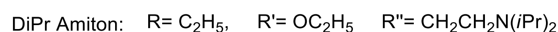
Scheme 6. Two possible major oxidation products from the herbicide dymron by laccase mediator system [205].

pling may play a major role in the oligomerization of oxidized isoproturon. The mechanism of oligomerization processes is depicted in Scheme **5**.

Maruyama *et al.* [189] reported the degradation of dymron, a common herbicide, by laccase-mediated oxidation. They got similar degradation products from HPLC and GCMS analysis that was previously identified by Uchiyama *et al.* [208] in the degradation of dymron in soil. In mass spectrometric analysis, two major fragments appeared at m/z 120 and m/z 146, corresponding to methylbenzyl moiety and phenylurea derivative Scheme **6**.

In contrast to the equivalent alkylthiol produced by the hydrolysis pathway, O-ethyl methylphosphonic acid and N,N-dialkylaminoethanesulfonate are produced during the oxidative hydrolysis of phosphonothiolates, according to Amitai *et al.* reported [209] (Scheme **7**). The result of alkylthiol hydrolysis is notorious because of the bad smell of mercaptane and moderate toxicity. As a result, the oxidative pathway produces less harmful and nontoxic products. For the successful degradation of toxic phosphorothiolates, fungal laccase was used in combination with several mediators. The rapid oxidative degradation of DiPr-Amiton occurred by laccase purified from Pleurotus ostreatus using ABTS as a mediator. This approach has a significant advantage over biocatalysis that overcomes the issue of some OP substrates having poor binding affinity.

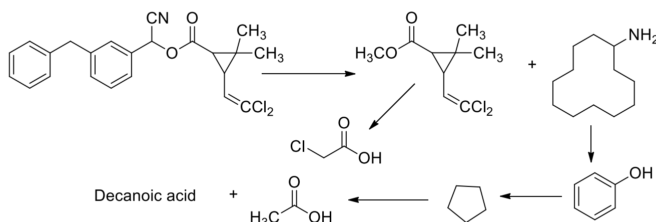




Scheme 7. Degradation pathways of DiPr-Amiton by hydrolysis and oxidation [209].

During the biodegradation study of cypermethrin, Gangola *et al.* proposed [210] a new pathway after analysing the intermediates after 15 days of incubation of bacterial strain 1D with cypermethrin, (Scheme **8**). The ester group of cy-

permethrin is first hydrolysed, which results in 3-(2, 2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate and cyclododecylamine. In the air, cyclododecylamine was unstable and oxidised to produce phenol, whereas 3-(2,2-dichloro-ethenyl)-2,2-dimethyl-cyclopropanecarboxylate hydrolyzed to generate chloroacetic acid. After that, phenol reacts with water and forms cyclopentanes, which further produces aliphatic compounds like acetic acid and decanoic acid.



Scheme 8. Proposed pathway of degradation of cypermethrin used by bacterial strain 1D [210].

CONCLUSION

The indiscriminate use of pesticides results in the accumulation of toxic compounds in the environment that must be removed or broken down into less toxic compounds. Removal of pesticides has gained priority as their presence is not desirable for food safety and compromises the health issues of both present and future generations. Laccases are one of the most important pesticide degrading enzymes due to their high specificity, broad substrate range, high efficiency, stability in different solvent systems, and ecological sustainability. Filamentous white rot fungi are advantageous for pesticide degradation over other organisms due to their extracellular production of a number of lignocellulolytic enzymes within which laccase is most important. Inadequate production, low stability, and restricted reusability are the foremost apprehension for the commercial application of enzymes. Therefore, emphasis should be given first to scaling up processes for the production of the enzyme laccase. Being a sustainable biocatalyst, an enormous number of laccases from various biological origins are essentially needed for a successful application, particularly for environmental issues like pesticide pollution. Various studies have concentrated on improved production of laccase in a cost-effective way while still raising questions vis-à-vis their industrial feasibility. Immobilization studies of laccases on various cost-effective support materials have improved the viability of the enzymes, particularly enhanced stability, easy recovery, and reusability. Although enzyme immobilization has a significant role in the degradation of pesticides, however possesses some drawbacks that restrict successful commercial relevance. In the future, the amalgamation of both rational and semi-rational approaches to genetic engineering using heterologous expression systems together with molecular mechanics/dynamics and quantum mechanics simulations will direct the development of high throughput technologies for the production of laccase and other pesticide degrading enzymes. These engineered enzymes like laccases can be efficiently utilized in pesticide bioremediation when immobilized on suitable career materials in the presence of appropriate mediators, thus approaching a green sustainable environment.

LIST OF ABBREVIATIONS

PAH = Polycyclic Aromatic Hydrocarbons
McoP = Multicopper Oxidase
OCPs = Organochlorine Pesticides
HCH = Hexachlorocyclohexanes
OPPs = Organophosphorus Pesticides
AChE = Acetylcholinesterase
GSH = Glutathione
PI = Peroxy Intermediate
NI = Intermediate
LMS = Laccase Mediator System
VLA = Violuric Acid

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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