

## Review Article

# Laccase: Microbial Sources, Production, Purification, and Potential Biotechnological Applications

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Laccase belongs to the blue multicopper oxidases and participates in cross-linking of monomers, degradation of polymers, and ring cleavage of aromatic compounds. It is widely distributed in higher plants and fungi. It is present in Ascomycetes, Deuteromycetes and Basidiomycetes and abundant in lignin-degrading white-rot fungi. It is also used in the synthesis of organic substance, where typical substrates are amines and phenols, the reaction products are dimers and oligomers derived from the coupling of reactive radical intermediates. In the recent years, these enzymes have gained application in the field of textile, pulp and paper, and food industry. Recently, it is also used in the design of biosensors, biofuel cells, as a medical diagnostics tool and bioremediation agent to clean up herbicides, pesticides and certain explosives in soil. Laccases have received attention of researchers in the last few decades due to their ability to oxidize both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant environmental pollutants. It has been identified as the principal enzyme associated with cuticular hardening in insects. Two main forms have been found: laccase-1 and laccase-2. This paper reviews the occurrence, mode of action, general properties, production, applications, and immobilization of laccases within different industrial fields.

## 1. Introduction

In the recent years, enzymes have gained great importance in Industries; laccases are one among them which are widely present in the nature. Laccases are the oldest and most studied enzymatic systems [1]. These enzymes contain 15–30% carbohydrate and have a molecule mass of 60–90 kDa. These are copper containing 1,4-benzenediol: oxygen oxidoreductases (EC 1.10.3.2) found in higher plants and microorganisms. These are glycosylated polyphenol oxidases that contain 4 copper ions per molecule that carry out 1 electron oxidation of phenolic and its related compound and reduce oxygen to water [2, 3]. When substrate is oxidized by a laccase, it loses a single electron and usually forms a free radical which may undergo further oxidation or nonenzymatic reactions including hydration, disproportionation, and polymerization [4]. These enzymes are polymeric and generally contain 1 each of type 1, type 2, and type 3 copper centre/subunit where the type 2 and type 3 are close together forming a trinuclear copper cluster.

Laccases are widely distributed in higher plants, bacteria, fungi, and insects. In plants, laccases are found in cabbages, turnip, potatoes, pears, apples, and other vegetables. They have been isolated from Ascomyceteous, Deuteromycteous and Basidiomycetous fungi to which more than 60 fungal strains belong [3]. The white-rot Basidiomycetes fungi efficiently degrade the lignin in comparison to Ascomycetes and Deuteromycetes which oxidize phenolic compounds to give phenoxy radicals and quinines [5].

Laccases play an important role in food industry, paper and pulp industry, textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutant and removal of endocrine disruptors [2]. These enzymes are used for pulp delignification, pesticide or insecticide degradation, organic synthesis [4], waste detoxification, textile dye transformation, food technological uses, and biosensor and analytical applications.

Recently laccases have been efficiently applied to nanobiotechnology due to their ability to catalyze electron transfer reactions without additional cofactor. The technique for

the immobilization of biomolecule such as layer-by-layer, micropatterning, and self-assembled monolayer technique can be used for preserving the enzymatic activity of laccases.

## 2. Sources of Laccases

Laccase is generally found in higher plants and fungi but recently it was found in some bacteria such as *S.lavendulae*, *S.cyanus*, and *Marinomonas mediterranea* [6–8]. In fungi, laccases appear more than the higher plants. Basidiomycetes such as *Phanerochaete chrysosporium*, *Theiophora terrestris*, and *Lenzites, betulina* [9], and white-rot fungi [10, 11] such as *Phlebia radiata* [12], *Pleurotus ostreatus* [13], and *Trametes versicolour* [14] also produce laccase. Many *Trichoderma* species such as *T. atroviride*, *T. harzianum* [15], and *T. longibrachiatum* [16] are the sources of laccases. Laccase from the *Monocillium indicum* was the first laccase to be characterized from Ascomycetes which shows peroxidase activity [8]. *Pycnoporus cinnabarinus* produces laccase as ligninolytic enzyme while *Pycnoporus sanguineus* produces laccase as phenol oxidase [17, 18]. In plants, laccase plays a role in lignification whereas in fungi it has been implicated in delignification, sporulation, pigment production, fruiting body formation, and plant pathogenesis [19, 20].

## 3. Mechanism of Laccases

The laccase catalysis occurs due to the reduction of one oxygen molecule to water accompanied with the oxidation of one electron with a wide range of aromatic compounds which includes polyphenol [21], methoxy-substituted monophenols, and aromatic amines [14]. Laccases contain 4 copper atoms termed Cu T1 (where the reducing substrate binds) and trinuclear copper cluster T2/T3 (electron transfer from type I Cu to the type II Cu and type III Cu trinuclear cluster/reduction of oxygen to water at the trinuclear cluster) [3]. These four copper ions are classified into three categories: Type 1 (T1), Type 2 (T2) and Type 3 (T3). These three types can be distinguished by using UV/visible and electronic paramagnetic resonance (EPR) spectroscopy.

At oxidizing state, the Type 1 Cu gives blue colour to the protein at an absorbance of 610 nm which is EPR detectable, Type 2 Cu does not give colour but is EPR detectable, and Type 3 Cu contains a pair of atoms in a binuclear conformation that give a weak absorbance in the near UV region but not detected by EPR signal [19]. The Type 2 copper and Type 3 copper form a trinuclear centre which is involved in the enzyme catalytic mechanism. The O<sub>2</sub> molecule binds to the trinuclear cluster for asymmetric activation, and it is postulated that the O<sub>2</sub> binding compartment appears to restrict the access of oxidizing agents. During steady state, laccase catalysis indicates that O<sub>2</sub> reduction takes place [3]. Laccase operates as a battery and stores electrons from individual oxidation reactions to reduce molecular oxygen. Hence, the oxidation of four reducing substrate molecules is necessary for the complete reduction of molecular oxygen to water. When laccase oxidizes the substrate, free radicals are generated. The lignin degradation proceeded by phenoxy

radical leads to oxidation at  $\alpha$ -carbon or cleavage of bond between  $\alpha$ -carbon and  $\beta$ -carbon. This oxidation results in an oxygen-centered free radical, which can be converted into a second enzyme-catalyzed reaction to quinone. The quinone and the free radicals can then undergo polymerization [19]. The organization of the copper sites in laccase is explained by the spectroscopic studies [22] which reveal that Type 2 copper coordinates two His-N and one oxygen atom as OH<sup>-</sup> while each copper of Type 3 coordinates three His residues. Further, both T2 and T3 copper sites have open coordination positions towards the center of trinuclear cluster with the negative protein compartment [23].

The laccase-mediated catalysis can be extended to non-phenolic substrates by the insertion of mediators. Mediators are low-molecular-weight organic compounds that are oxidized by laccase. The highly active cation radicals oxidize the non-phenolic compounds that laccase alone cannot oxidize. The most common synthetic mediators are 1-hydroxy benzotriazole (HOBT), N-hydroxyphthalimide (NHPI), 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), and 3 hydroxyanthranilic acid [14, 24]. In presence of ABTS oxygen uptake by laccase is faster than the HOBT.

## 4. Properties of Laccase Enzyme

Laccases are mainly monomeric, dimeric, and tetrameric glycoprotein. Glycosylation plays an important role in copper retention, thermal stability, susceptibility to proteolytic degradation, and secretion. Upon purification, laccase enzymes demonstrate considerable heterogeneity. Glycosylation content and composition of glycoprotein vary with growth medium composition.

## 5. Production of Laccase

Laccases are the enzymes which are secreted out in the medium extracellularly by several fungi [25] during the secondary metabolism but not all fungal species produce laccase such as Zygomycetes and Chytridiomycetes [26]. The literature describes the production of laccase by soil as well as some freshwater Ascomycetes species [27–31]. In addition to this, laccase production was also found in *Gaeumannomyces graminis*, *Magnaporthe grisea*, *Ophiostoma novo-ulmi*, *Marginella*, *Melanocarpus albomyces*, *Monocillium indicum*, *Neurospora crassa*, and *Podospora anserina* [8, 32–38].

*Botryosphaeria* produces a dimethoxyphenol oxidizing enzyme which is a true laccase [39]. The Ascomycetes species which participate in the plant biomass decay contain laccase genes which oxidize syringaldazine [40]. *Cryptococcus neoformans* is Basidiomycetes yeast which produces laccase and oxidizes phenols and aminophenol but is unable to oxidize tyrosine [1]. Only plasma membrane-bound multicopper oxidase of *Saccharomyces cerevisiae* shows homology with fungal laccase [41].

Basidiomycetes and Saprotrophic fungi are the most widely known species that produce substantial amount of laccase in changeable quantity [42]. In case of *Pycnoporus cinnabarinus*, laccase was the only ligninolytic enzyme which

degrades lignin [17]. But the laccase producing capability of brown-rot fungi is not known, and no laccase has been purified. Recently it was found that brown-rot fungus *Coniophora puteana* [43] oxidizes the syringaldazine and supports the oxidation of ABTS in *Laetiporus sulphureus* [44]. Several factors influence laccase production such as type of cultivation (submerged or solid state), carbon limitation, and nitrogen source [45].

## 6. Influence of Carbon and Nitrogen Source

The organism grown in the defined medium contains 0.1% w/v yeast extract and 1% (w/v) different carbon sources as well as nitrogen sources. Glucose, mannose, maltose, fructose, and lactose are the commonly used carbon sources. The excess glucose and sucrose reduce the production of laccase by obstructing the initiation. This problem of production of enzyme can be improved by using polymeric substrates like cellulose [43]. Yeast extract, peptone, urea,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{NaNO}_3$  are the commonly used nitrogen sources. Laccase production is triggered by nitrogen depletion [46] but some nitrogen strains do not affect the enzyme activity [47]. Some studies show that the elevated laccase activity was achieved by using low carbon-to-nitrogen ratio [48] while others show that it was achieved at high carbon-to-nitrogen ratio [49].

## 7. Influence of Temperature

The effect of temperature is limited in case of laccase production. The optimal temperature of laccase differs greatly from one strain to another. It has been found that 25°C is the optimal temperature for laccase production in presence of light, but, in case of dark, the optimal temperature is 30°C [19]. The optimum temperature range for laccase production is between 25°C and 30°C [50]. Farnet et al. [51] found that preincubation of enzymes at 40°C and 50°C greatly increased laccase activity. The laccase from *P. ostreatus* is almost fully active in the temperature range of 40°C–60°C, with maximum activity at 50°C. The activity remains unaltered after prolonged incubation at 40°C for more than 4 h [52]. Nyanhongo et al. [53] showed that laccase produced by *T. modesta* was fully active at 50°C and was very stable at 40°C but half-life decreased to 120 min at higher temperature (60°C).

## 8. Influence of pH

The effect of pH is limited in case of laccase production [19]. The optimum value of pH varies according to the substrate because different substrate causes different reaction for laccases. Many reports suggested that the bell-shaped profile occurs in case of laccase activity. At high pH value, the potential difference between the phenolic substrate and the T1 copper can increase the substrate oxidation while the hydroxide anion ( $\text{OH}^-$ ) binds to the T2/T3 copper centre. These effects help us in determining the optimum value of pH for laccase enzyme [54]. Cordi et al. [55] use syringaldazine as a substrate and determine the effect of pH

on enzyme activity in the range of 3.0–8.0. The optimum pH for L1 (isozyme of laccase) was 4.0 whereas the optimum pH for L2 was 5.0. Han et al. [56] extracted laccase from *Trametes versicolour* which showed high enzyme activity at broad range of pH and temperature ranges but the optimum activity was found at pH 3.0 and 50°C temperature. Laccase extracted from *Stereum ostrea* showed the highest activity at pH 6.0 and 40°C temperature [57]. When fungi are grown in the medium of pH 5.0, the laccase will produce in excess but most studies show that pH between 4.5 and 6.0 is suitable for enzyme production [19].

## 9. Influence of Agitator

Agitation is another factor which affects laccase production. Hess et al. [58] found that mycelia are damaged when fungus is grown in the stirred tank reactor and laccase production by *Trametes multicolour* is considerably decreased. Mohorčić et al. [59] found that cultivation of white-rot fungus *Bjerkandera adusta* in a stirred tank reactor with very low activities was attained. Tavares et al. [60] observed that agitation did not play any role in the production of laccase by *T. versicolour*.

## 10. Influence of Inducer

Laccase production has been seen to be highly dependent on fungus cultivation [61]. During secondary metabolic phase, ligninolytic systems are activated and triggered by nitrogen concentration [49]. Laccases are generally produced in low concentrations by laccase-producing fungi [39], but higher concentrations were obtained with the addition of various supplements such as xenobiotic compound to media [62, 63]. The addition of aromatic compounds such as 2,5-xylydine, lignin, and veratryl alcohol is known to increase and induce laccase activity [63, 64]. Veratryl alcohol is an aromatic compound; its addition to cultivation media results in an increase of laccase production [65]. The addition of 2,5-xylydine after 24 h of cultivation gave the highest induction of laccase activity and increased laccase activity ninefold. At higher concentrations the 2,5-xylydine had a reducing effect due to toxicity [17]. The promoter region encoding for laccase contains various recognition sites that are specific for xenobiotics and heavy metals [66]; they bind to the recognition sites and induce laccase production. The addition of inducer increases the concentration of a specific laccase enzyme [67]. Lee et al. [62] found that alcohol enhanced laccase activity more in comparison to xylydine. This is a very economical way to enhance laccase production. Cellobiose increase laccase activity by profusing branch in certain *Trametes* species [68]. Low concentrations of  $\text{Cu}^{+2}$  to the cultivation media increases the laccase production 50 times in comparison to basal medium [13, 69]. A new basidiomycete, *Trametes* sp. 420, produced laccase in glucose medium and in cellobiose medium with induction by 0.5 mM and 6 mM o-toluidine [70]. D'Souza-Ticlo et al. [71] performed various experiments to determine the effect of inhibitor on the activity of Lac-II in the presence of sodium

azide, SDS, and mercaptoethanol. They found that 32–37% activity of Lac-II was inhibited in the presence of Sn, Ag, and Hg while 56% and 48% Lac-II activity was inhibited in the presence of Cr and W, respectively. Dubé et al. [72] found that 5Mm EDTA inhibits the total laccase activity.

## 11. Type of Cultivation

Submerged and solid-state modes of fermentation are used intensely for the production of laccase. Wild-type filamentous fungi are used for large-scale production of laccase in different cultivation techniques.

**11.1. Submerged Fermentation.** Submerged fermentation involves the nurturing of microorganisms in high oxygen concentrated liquid nutrient medium. Viscosity of broth is the major problem associated with the fungal submerged fermentations. When fungal cell grows, mycelium is formed which hinders impeller action, due to this limitation occurring in oxygen and mass transfer. For dealing with this problem, different strategies have been employed. Bioreactor operates in continuous manner for obtaining high efficiency. In this *Trametes versicolour* is employed which decolorizes the synthetic dye, and for this purpose pulsed system has been developed [73–77]. Broth viscosity, oxygen, and mass transfer problems are also solved by cell immobilization. Luke and Burton [78] reported that continuous laccase production takes place without enzyme deactivation for a period of 4 months due to the immobilization of the *Neurospora crassa* on membrane. For bioremediation of pentachlorophenol (PCP) and 2,4-dichlorophenol (2,4 DCP), nylon mesh is used for comparing the free cell culture of *T. versicolour* with immobilized cultures. Couto et al. and Sedarati et al. investigated that, in fixed bed bioreactors, stainless steel showed the highest laccase activity among different synthetic materials which were used as carriers for the immobilization of *Trametes hirsute* [79–81]. The most effective way of producing laccase is Fed-batch operation through which the highest laccase activity can be obtained.

**11.2. Solid-State Fermentation.** SSF is suitable for the production of enzymes by using natural substrates such as agricultural residues because they mimic the conditions under which the fungi grow naturally [82–85]. The lignin, cellulose and hemicelluloses are rich in sugar and promote fungal growth in fermentor and make the process more economical [86]. The major drawback is the bioreactor design in which heat and mass transfer is limited. Different bioreactor configurations have been studied for laccase production such as immersion configuration, expanded bed, tray, inert (nylon) and noninert support (barley bran) in which tray configuration gave the best response [87]. A tray and immersion configuration is compared for laccase production by using grape seeds and orange peel as substrate [88, 89].

Laccase production by both solid-state and submerged fermentation is higher in case of rice bran than other substrates. The rice bran inductive capability is based on the

phenolic compounds such as ferulic acid, and vanillic acid which induce the laccase production [90]. Many agricultural wastes such as grape seeds, grape stalks, barley bran [91], cotton stalk, molasses waste water [92] and wheat bran [93] are also used as substrate for laccase production. However, laccase production in both solid-state and submerged fermentation did not reach up to the maximum level; that is why prolonged cultivation is required.

## 12. Purification of Laccase

Ammonium sulphate is being commonly used for the enzyme purification for many years. But researchers have found much more efficient methodologies such as protein precipitation by ammonium sulphate, anion exchange chromatography, desalt/buffer exchange of protein, and gel filtration chromatography. Single-step laccase purification from *Neurospora crassa* takes place by using celite chromatography and 54 fold purification was obtained with specific activity of 333 U mg<sup>-1</sup> [94]. Laccase from LLP13 was first purified with column chromatography and then purified with gel filtration [10, 11]. Laccase from *T. versicolour* is purified by using ethanol precipitation, DEAE-Sepharose, Phenyl-Sepharose and Sephadex G-100 chromatography which is a single monomeric laccase with a specific activity of 91,443 U mg<sup>-1</sup> [58]. Laccase from *T. versicolour* is purified with Ion Exchange chromatography followed by gel filtration with specific activity of 101 U mL<sup>-1</sup> and 34.8-fold purification [55]. Laccase from *Stereum ostrea* is purified with ammonium sulphate followed by Sephadex G-100 column chromatography with 70-fold purification [9]. Laccase from fruiting bodies is purified with ammonium sulphate precipitation with 40–70% saturation and DEAE cellulose chromatography then 1.34 and 3.07 fold purification is obtained respectively [95].

## 13. Applications of Laccase

Laccase is important because it oxidizes both the toxic and nontoxic substrates. It is utilized in textile industry, food processing industry, wood processing industry, pharmaceutical industry, and chemical industry. This enzyme is very specific, ecologically sustainable and a proficient catalyst. Applications of laccase are as follows.

**13.1. Dye Decolorization.** Textile industry utilizes large volume of water and chemicals for wet processing. These chemicals range from inorganic compounds to organic compounds. The chemical structure of dyes provides a resistance to fading when exposed to light, water, and other chemicals. Laccase degrades dye; that is why laccase-based processes have been developed which include synthetic dyes and are being used in the industry nowadays [96, 97].

Blánquez et al. [98] used *T. versicolour* in the form of pellets to treat a black liquors discharge for detoxifying and reducing the colour, aromatic compounds, and chemical oxygen demand (COD). They found that colour and aromatic compounds were reduced up to 70–80% and COD

was reduced up to 60%. They concluded that *T. versicolor* is able to produce laccase. *T. versicolor* completely decolorizes the Amaranth, Tropaeolin O, Reactive Blue 15, Congo Red, and Reactive Black 5 with no dye sorption while it partially decolorizes Brilliant Red 3G-P, Brilliant Yellow 3B-A and Remazol Brilliant Blue R with some dye sorption. They found that after decolorization, toxicity of few dyes remained the same while some became nontoxic [99]. Laccase-based hair dyes are less irritant and easier to handle than conventional hair dyes because laccases replace  $H_2O_2$  in the dye formulation [100]. Laccase are also used in dechlorination process. Xylidine is a laccase inducer which increases dechlorination activity due to which dissolved oxygen concentration is reduced [101]. Romero et al. [77] found that bacteria *S. maltophilia* decolorizes some synthetic dyes (methylene blue, methyl green, toluidine blue, Congo red, methyl orange, and pink) as well as the industrial effluent.

**13.2. Bioremediation and Biodegradation.** Due to rapid industrialization and extensive use of pesticides for better agricultural productivity, contamination of soil, water, and air take place which is a serious environmental problem of today. Polychlorinated biphenyls (PCB), benzene, toluene, ethyl benzene, xylene (BTEX), polycyclic aromatic hydrocarbons (PAH), pentachlorophenol (PCP), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), and trinitrotoluene (TNT) are the substances which are known for their carcinogenic as well as mutagenic effect and are persistent in the environment. Fungi renovate a wide variety of hazardous chemicals; that is why the researcher's interest is generated in them [102].

*T. versicolor* is used for the bioremediation of atrazine in soil with low moisture and organic contents that are normally found in semiarid and Mediterranean-like ecosystems [103]. Keum and Li [104] obtained laccase from *T. versicolor* and *Pleurotus ostreatus* for the degradation of PCBs as well as phenol and found as chlorination increases, degradation rate decreases and concluded that 3-hydroxy biphenyl was more resistant to laccase degradation than 2- or 4-hydroxy analogues. After five days of incubation, when glucose and fructose were used as a cosubstrate than 71% of p-hydroxy benzoic acid and 56% of protocatechuic acid were degraded [105].

Laccase obtained from *T. villosa* remediates the soil by degrading 2,4-DCP (2,4-dichlorophenol). An experiment was performed by Ahm in which he took 2 types of soil: in soil 1, both free and immobilized laccase remove 100% of 2,4-DCP (without regard of moisture content). In soil 2, immobilized laccase removed more 2,4-DCP (about 95%) than free enzyme (55%, 75%, and 90%, at 30%, 55%, 100% maximum water holding capacity) [106]. *Cerrena unicolor* produces laccase in the low nitrogen medium which has the capability of reducing lignin content from sugarcane bagasse up to 36% within 24 h at 30°C [71].

**13.3. Paper and Pulp Industry.** Chlorine and oxygen-based chemical oxidants are used for the separation and degradation of lignin which is required for the preparation of paper

at industrial level. But some problems such as recycling, cost, and toxicity remain unsolved. However, in the existing bleaching process, LMS could be easily implemented because it leads to a partial replacement of  $ClO_2$  in pulp mills [54].

**13.4. Food Processing Industry.** In food industry, laccase is used for the elimination of undesirable phenolic compound in baking, juice processing, wine stabilization, and bioremediation of waste water [2]. Laccase improves not only the functionality but also the sensory properties [107]. In beer industry, laccase not only provides stability but also increases the shelf life of beer. In beer, haze formation takes place which is stimulated by the naturally present proanthocyanidins polyphenol and is referred to as chill haze. At room temperature or above, warming of beer can redissolve the complex. After certain periods of time, phenolic rings are replaced by the sulphhydryl group and permanent haze is formed which cannot be redissolved. For polyphenol oxidation, laccase has been used which is capable of removing the excess oxygen and also due to which the shelf life of beer increases [108, 109]. For making a fruit juice stable, laccase is commonly used. Phenol compounds and their oxidative products present naturally in the fruit juice give colour and taste to the juice. Colour and aroma change when polymerization and oxidation of phenolic and polyphenol take place. These changes are due to the high concentration of polyphenol and referred to as enzymatic darkening [110]. Laccase treatment removes phenol as well as substrate-enzyme complex by the help of membrane filtration, and colour stability is achieved, although turbidity is present. Laccase treatment is more effective in comparison to conventional methods. For improving the texture, volume, flavor and freshness of bread, wide range of enzymes are used. When laccase is added to the dough, strength of gluten structures in dough and baked products is improved: product volume increases, crumb structure improves, and softness of baked products takes place. Due to the laccase addition, stickiness decreases, strength and stability increase and the ability of machine is also improved which can also be seen by using a low-quality flour [109]. At crushing and pressing stage, the high concentration of phenolic and polyphenolic compound play an important role in the wine production. The high concentration of polyphenol obtained from the stems, seeds and skins which depends on the grape variety and vinification conditions contributes to of colour and astringency [111]. Due to the complex event, polyphenol oxidation occurs in musts and wines resulting in the increase in colour and flavour change which is referred to as maderization [108]. Catalytic factors, polyphenol removal, clarification, polyvinylpyrrolidone (PVPP), and high doses of sulfur dioxide are utilized to prevent maderization. Minussi et al. found that polyphenol removal is selective and results in undesirable organoleptic characteristics and concluded that laccase treatment is feasible, increasing storability and reducing processing costs [111].

**13.5. Other Applications.** Laccase not only is used in food industry, paper and pulp industry, textile industry but also has

other applications. In traditional system, PVPP is used for the removal of excess polyphenol which has low biodegradability and creates problems in wastewater treatment [109]. Laccase has the ability to decrease odour arising from the garbage disposal sites, livestock farms and pulp mills. Since laccases catalyze the electron transfer reactions without additional cofactors, they can also be used as biosensors to detect various phenolic compounds, oxygen, and azide. As biosensor, laccase can detect morphine, codeine, catecholamine, estimate phenol or other enzymes in fruit juice and plant flavonoid. Recently, laccase has been used as a biocatalyst for the synthesis of organic substance as well as in the design of biofuel cell [54]. For the bioremediation of food industry wastewater, laccase has been utilized. In bioremediation process, contaminants are biotransformed to their original status which has no bad effects on the environment [112]. Large amount of polyphenol is present in the beer factory wastewater which is dark brown in colour and degraded by the white-rot fungus *Coriolopsis gallica* [113]. Laccase produced from *Trametes* sp. bioremediates the distillery wastewater generated from the sugarcane molasses fermentation with high content of organic matter [114]. Olive mill wastewater is bioremediated by the help of immobilized laccase which is beneficial for the cultivation of fungi for laccase production [109]. Many countries pose some rules and regulation for the pollutants which includes phenols and amines [115].

**13.6. Laccase Function in Insects.** Laccase has been found in the cuticles of many insect species [116, 117] and is involved in cuticle sclerotization [118, 119]. Laccases oxidizes catechols in the cuticle to their corresponding quinones, which catalyzes protein cross-linking reactions. In several holometabolous insects, laccase has been identified as the principal enzyme associated with cuticular hardening [120–123]. The insect laccase is a long amino-terminal sequence characterized by a unique domain consisting of several conserved cysteine, aromatic, and charged residues. In recent years, cloning of insect laccase genes has been performed [120, 121, 124] and two main forms have been found: laccase-1 and laccase-2 [120, 121, 125, 126]. Laccase-1 was found to be expressed in the midgut, Malpighian tubules [121, 126, 127] and fat body as well as the epidermis of the tobacco hornworm, *Manduca sexta*, and may oxidize toxic compounds ingested by the insect [121]. On the other hand, laccase-2 was involved in cuticle tanning of the red flour beetle, *Tribolium castaneum* [120]. Recently, a laccase in the salivary glands of *N. cincticeps* was identified, which is secreted in watery saliva, by using biochemical and histochemical approaches [128]. A possible function of salivary laccase (diphenoloxidase) is in the enhancement of the oxidative gelling occurring in the stylet sheath by a quinone-tanning reaction [129] and rapid oxidation of potentially toxic monolignols to nontoxic polymers during feeding [128].

## 14. Laccase Immobilization

When enzyme is immobilized, it becomes more vigorous and resistant to alteration in environment which allows

easy recovery and reuse of enzyme for multiple purposes. That is why researchers are moving towards the efficient methods of immobilization which influence the properties of the biocatalyst. Laccase immobilization has been studied with a wide range of different immobilization methods and substrates.

Laccase produced by *Trametes versicolour* is immobilized on silica which is chemically modified with imidazole groups and Amberlite IRA-400. Glass-ceramic is chemically modified by carbodiimide/glutaraldehyde as well as aminopropyltriethoxysilane/glutaraldehyde, and montmorillonite is modified by aminopropyltriethoxysilane/glutaraldehyde which was used in the decolorization of textile dyes [130]. Laccase can be immobilized on different pyrolytic graphite (best support), ceramics supports and on a carbon fiber electrode where it acts as biosensor. At the 12th day, maximum laccase activity  $40,774.0 \text{ U L}^{-1}$  was achieved [131]. An optical biosensor is fabricated by using stacked films for the detection of phenolic compounds; 3-methyl-2-benzothiazolinone hydrazone (MBTH) was immobilized on a silicate film and laccase on a chitosan film [132].

## 15. Conclusion

Laccases are the versatile enzymes which catalyze oxidation reactions coupled to four-electron reduction of molecular oxygen to water. They are multicopper enzymes which are widely distributed in higher plants and fungi. They are capable of degrading lignin and are present abundantly in many white-rot fungi. They decolorize and detoxify the industrial effluents and help in wastewater treatment. They act on both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant environmental pollutants which help researchers to put them in various biotechnological applications. They can be effectively used in paper and pulp industries, textile industries, xenobiotic degradation, and bioremediation and act as biosensor. Laccase has been applied to nanobiotechnology which is an increasing research field and catalyzes electron transfer reactions without additional cofactors. Recently several techniques have been developed for the immobilization of biomolecule such as micropatterning, self-assembled monolayer, and layer-by-layer technique which immobilize laccase and preserve their enzymatic activity. Hence laccase is receiving much attention of researchers around the globe.

## 16. Future Trends and Perspectives

This paper shows that laccase has a great potential application in several areas of food industry. However, one of the limitations for the large-scale application of laccase is the lack of capacity to produce large volumes of highly active enzyme at an affordable cost. The use of inexpensive sources for laccase production is being explored in recent times. In this regard, an emerging field in management of industrial wastewater is exploiting its nutritive potential for production of laccase. Besides solid wastes, wastewater from the food processing industry is particularly promising for that.

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