

# Laccase as Key Enzyme Among: Fungi-Pollutant-Environment and Sustainable Development Goals Nexus

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## ABSTRACT

Pollution from industrial development has led to the production of various deadly substances of refractory classes such as polycyclic aromatic hydrocarbons, toxic dyes, pesticides, and heavy metals. Their toxicity and mutagenicity affect humans, plants, and aquatic organisms. Biodegradation, of course, takes place by fungi, which means that complex organic compounds are broken down into simpler inorganic forms. Although these organisms utilize these organisms as an energy source, bioremediation is a human-designed technology that uses microbes through techniques of natural attenuation, biostimulation, or bioaugmentation to enhance their capabilities to reduce pollutants. Various microorganisms such as bacteria, fungi, algae, and protozoa can degrade environmental contaminant with promising capacity. In order for degrading fungi to achieve the highest biodegradation rate under optimal conditions, certain parameters must be set. These factors are biological factors such as bioavailability, nutrient availability, crop type and microbial type, and environmental factors such as pH, temperature, oxygen availability and contaminant concentrations. The biodegradation mechanism mainly depends on microbial enzymes such as oxidoreductases, hydrolases, peroxidases, oxygenases, proteases, lipases and varnishes. Genetically engineered microbes are used to further enhance contaminant remediation, ensuring safe biodegradation by commensal microbes.

**Keywords:** Microorganisms; Biodegradation; Sustainable development goals; Ligninolytic Enzymes; Laccase

**Abbreviations:** SDGs: Sustainable Development Goals; LAC: Laccase; LIP: Lignin Peroxidase; MNP: Manganese Peroxidase; VP: General Peroxidase; WRF: White Rot Fungi; EPR: Electron Paramagnetic Resonance; COD: Chemical Oxygen Demand; BOD: Biological Oxygen Demand; LMEs: Lignin-Modifying Enzymes

## Introduction

Nowadays, environmental problems are increasing rapidly; unresolved problems arising from environmental pollution explain the inability to control local problems. Globally, the environment is always at risk from climate disturbances such as unexpected, devastating heavy rains and recurrent flooding from intense tropical storms. Environmental problems have become intense due to uncontrolled human activity [1]. In general, pollutants are classified according to their physical or chemical properties. First: Chemical pollutants are the result of man-made factors such as toxic substances and nuisances such as plastic or glass, and natural factors including critical pollutants such as carbon and nitrate also include minor pollutants such as lead and copper. Second: Energy and pollutants are the result of heat, noise and ionizing radiation [2]. Fungi are known for their ability to produce a wide variety of extracellular enzymes. The major organisms responsible for lignocellulose degradation are aerobic filamentous fungi, the most rapidly degrading of this group being the basidiomycetes [2-4]. Wood-rotting basidiomycetes are generally classified as white-rot fungi, brown-rot fungi, and litter rot fungi. The only organisms that can efficiently mineralize lignin are the Basidiomycota white rot fungi and related waste-decomposing fungi [5-7].

## Discussion

### a. Microbial Degradation of Lignin

White rot fungi (WRF) belonging to the Basidiomycota family produce different isoforms of extracellular ligninolytic enzymes: laccase (Lac), lignin peroxidase (LiP), manganese peroxidase (MnP) and general peroxidase (VP). Various peroxidases, including the latter shares the catalytic properties of LiP and MnP [8-10,3]. These enzymes are not only directly involved in the degradation of lignin in natural lignocellulosic substrates, but also the degradation of various xenobiotic compounds, including dyes [11-13]. Unlike most fungi and bacteria, white rot fungi can completely break down lignin to carbon dioxide and water. This species is widely distributed and is found in both tropical and temperate environments. White-rot fungi are also well-adapted to digest other plant materials, and species vary greatly in their relative efficiencies in cellulolytic and lignolytic degradation [1,7,14].

### b. Ligninolytic Enzymes

Three major classes of extracellular enzymes called MnP, LiP, and laccases are thought to be important in the fungal degradation of lignin [15,4]. Because these enzymes are non-specific, they can also target a wide variety of refractory compounds, including several herbicides structurally related to lignin. Such herbicides accumulate in soil and water, generally as a result of poor chemical management by farms, industry and society [16]. Purified laccase, lignin peroxidase, and manganese-dependent peroxidase are

potential enzymes for various industrial applications [9]. LiP and MnP are heme proteins, whereas laccase is a copper-containing protein. Some wood-degrading fungi contain all three classes of lignin-modifying enzymes (LMEs), while others contain only one or two of these enzymes [15].

### c. Degradation and Oxygen Availability of Polycyclic Aromatic Hydrocarbons (PAHs)

The degradation of PAHs depends on the ability of microorganisms to introduce oxygen into the rings, resulting in both PAH solubility and chemical reactivity. Increases [4]. In contrast, PAHs accumulate in areas with little or no oxygen, and degradation is greatly reduced under these conditions compared to aerobic conditions, where oxygen is used for the initial cleavage of these compounds. Indicates that Microorganisms that can degrade PAHs in hypoxic regions use non-oxygen terminal electron acceptors. These microorganisms can be divided into facultative anaerobes (reduce nitrate, iron and manganese) and obligate anaerobes (reduce sulfate); [17].

### d. Applications of Ligninolytic Enzymes

Due to their low specificity, ligninolytic enzymes have become the subject of intensive research for a variety of practical applications. Some practical applications include biotransformation of lignocellulosic biomass into feed, fuels and chemicals, removal of xenobiotics from water streams [18], dioxins, polychlorinated biphenyls, various Degradation of highly toxic environmental chemicals such as dye contaminants, polyaromatic hydrocarbons [9], Removal of phenolic compounds from wine, biosensors, dye decolorization [2,19], wastewater contaminated with industrial waste drug analysis, Ethanol production [20], biopulping, bioleaching of paper pulp, and removal of lignin from wood tissue.

### e. Laccase

Laccase (Lac, EC1.10.3.2), (benzenediol:oxygen oxidoreductase), performs the single-electron oxidation of phenols, aromatic amines, and other electron-rich substrates Blue copper oxidase that catalyzes, [21]. They oxidize a wide range of reducing substrates, including phenolic compounds and aromatic amines, donating electrons to dioxygen and producing water [22]. Laccase can only directly oxidize the smaller lignin phenol units, whereas the native phenol [23], significantly enhancing oxidative power. However, due to the high redox potential of non-phenolic moieties and the size of lignin polymers, efficient lignin degradation requires the concerted action of laccase and small redox mediators [9]. Laccase has been isolated from bacteria, fungi, higher plants, and insects [14]. They are fungal pathogenicity conidial pigmentation timberification biofuel production [18], and crop protection However, their most important role is played by lignin degradation

and humus processes [24]. The wide range of substrates that laccase can attack has led to the search for new enzyme sources. Laccases produced by white rot fungi oxidize aromatic contaminants such as chlorophenols, PAHs and dyes and are therefore widely used in various detoxification processes [25].

### Molecular Properties of Laccase

Laccases are glycosylated monomeric or homodimeric proteins that generally have less sugar linkage (10-25%) than plant enzymes in fungi and bacteria. Carbohydrate compounds include monosaccharides such as hexosamine, glucose, mannose, galactose, fucose, and arabinose [26]. By SDS-PAGE, most laccases exhibit mobilities corresponding to molecular weights of 60-100 kDa, of which 10-50% can be attributed to glycosylation. Mannose is one of the major carbohydrate components bound to laccase. Laccase glycosylation is involved in secretion, proteolytic sensitivity, activity, copper retention, and thermostability [27]. Most of the laccases studied are extracellular proteins, but intracellular laccases have been identified in some fungi and insects. The isoelectric points (pI) of fungal laccases range from 3 to 7, whereas plant laccases have pI values of up to 9. The main difference between the two enzymes is that the fungal enzyme has a pH optimum between pH 3.6 and 5.2, whereas the *Rhus vernicifera* laccase has an optimum pH between 6.8 and 7.4. Although fungal enzymes may have lower pH optima due to their suitability for growth under acidic conditions, the pH optima of intracellular plant laccases are closer to the physiological range. Therefore, differences in optimal pH may be due to differences in physiological function.

In addition to changing pH, these enzymes also differ in function. Fungal enzymes are responsible for removing toxic phenols from the medium in which these fungi grow under natural conditions, whereas plant enzymes are involved in synthetic processes such as lignin formation [28]. Purified laccase exhibits a characteristic blue color appearance based on electronic absorption near 600 nm. A typical UV-visible spectrum of laccase (resting state) exhibits two maxima near 280 nm and 600 nm and a shoulder near 330 nm. The ratio of absorbance at 280 nm to 600 nm is typically 14-30 [29]. The laccase absorption at 330 nm compared to 600 nm is 0.5-2. In the holoenzyme form, most laccases have four copper atoms per monomer [14], whereas *Phlebia* laccases have two copper and one pyrroloquinoline quinone prosthetic group. It has been reported that these copper atoms are classified into three groups using UV/visible and electron paramagnetic resonance (EPR) spectroscopy.

### Function of Laccase

In some fungi, the laccase reaction is independent of lignin degradation. Laccase plays a role in the morphogenesis and differentiation of sporulating and dormant structures in basidiomycetes and wood lignin biodegradation in white-rot

fungi [27]. Laccase is involved in mycelium and fruiting body pigmentation, improves cell-cell adhesion, aids in rhizome formation, and is also involved in the formation of the polyphenolic glue that binds the hyphae together. Various plant pathogens also produce extracellular laccases that enable fungi to overcome host immune responses [30]. Laccase also promotes plant tissue detoxification through oxidation of antifungal phenols or inactivation of phytoalexins. Laccases have been hypothesized to be involved in a variety of cellular and microbial activities. Recent studies on the physiological functions of laccase include studies on plant cell wall biosynthesis, phytopathogenicity, woody material degradation and humus, insect hardening, bacterial melanization, and melanin-associated toxicity to humans [24].

### Isozymes

Many laccase-producing bacteria secrete isoforms of the same enzyme. These isoenzymes have been shown to be derived from the same or different genes encoding laccase enzymes. The number of isozymes present varies between and within species, depending on whether they are induced or not. Isozymes can vary greatly in their stability, pH and temperature optima, and affinities for different substrates [31]. Furthermore, different isoenzymes may play different roles in the physiology of different species or in the same species under different conditions. Gene sequences encoding various laccases have been reported from many lignin-degrading bacteria. These sequences encode proteins between amino acid residues 515 and 619, and the phylogenetic closeness between them is shown by sequence comparison [32].

### Mechanism of Action of Laccase

Laccase attacks only the phenolic subunit of lignin (Archibald et al. 1997), causing  $\alpha$  oxidation,  $\alpha$ -C $\beta$  cleavage, and aryl-alkyl cleavage. Laccase catalysis is thought to involve:

- Reduction of type 1 copper by reduction of the substrate.
- Internal electron transfer from type 1 to copper type 2 and type 3.
- Reduction of oxygen to water at type 2 and type 3 copper sites.

Oxidation of reduced substrates by laccase usually involves the loss of a single electron and the formation of free (cation) radicals. Radicals are generally unstable and may undergo further laccase-catalyzed oxidation (e.g., to form quinones from phenols) or non-enzymatic reactions (e.g., hydration, disproportionation or polymerization) [33]. Laccase resembles other phenol oxidases that preferentially polymerize lignin by coupling phenoxy radicals generated by the oxidation of lignin's phenolic groups. However, the substrate range of laccase is 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) [33].

## Purification and Characterization of Laccase

In general, plant laccases are purified from sap or tissue extracts, whereas extracellular fungal laccases are purified from the media (fermentation broth) of the organism of choice. Various protein purification techniques are commonly used to purify laccase. Typical purification protocols include ultrafiltration, ion exchange, gel filtration, hydrophobic interaction, or other electrophoretic and chromatographic techniques.

**Effect of Ph Value on Laccase Activity the Optimum Ph for:** Laccase is highly substrate dependent. For phenols, the pH optimum ranges from 3 to 7 for fungal laccases and up to 9 for plant laccases. When using ABTS as a substrate, the optimum pH is more acidic, ranging from pH 3 to pH 5. In general, laccase activity has a bell-shaped profile and pH optima vary widely [14]. This variation may be due to changes in response caused by substrates, oxygen, or the enzyme itself [28].

**Effect of Temperature on Laccase Activity:** The optimum temperature for laccase varies greatly between strains. Laccase isolated from *Ganoderma lucidum* showed a temperature optimum of 20–25 °C and was stable at 10–50 °C for 4 hours. Laccase isolated from *Marasmius quercophilus* was shown to be stable at 60°C for 1 hour. Other reports further found that pre-incubation of the enzyme at 40°C and 50°C significantly increased laccase activity [34].

**Effect of Inhibitors on Laccase Activity:** In general, laccase responds similarly to several inhibitors of enzymatic activity; [35]. We found that azide, thioglycolate, and diethyldithiocarbamate all inhibited laccase activity, but EDTA had little effect on laccase activity. Small anions such as halides (except iodide), azide, cyanide, and hydroxide bind to laccase, leading to disruption of internal electron transport and inhibition of activity. Other inhibitors include metal ions (such as Hg<sup>2+</sup>), fatty acids, sulfhydryl reagents, hydroxylglycine, kojic acid, and cationic quaternary ammonium detergents [14].

## Uses of Microbial Ligninolytic Enzymes

Ligninolytic Enzymes have many biotechnological applications due to its ability to oxidize a wide range of phenolic and non-phenolic compounds. Laccase has many uses in agriculture, medicine and industry [18]. Laccase is also used to purify water in many purification systems. Applications of laccase mainly include the purification of industrial wastewater from industries such as the paper, pulp, textile and petrochemical industries. Laccase is also used in medical diagnostics and to remove herbicides, pesticides, and some explosives from soil. It also has medical uses for making certain pharmaceuticals, such as anticancer drugs, and is added to cosmetics to minimize toxic effects. Laccase is used for many bioremediation purposes because of its extraordinary ability to remove xenobiotics and generate polymeric products [36].

## Microbial Enzymes Applications in the Food Industry:

**Wine Stabilization:** Ligninolytic Enzymes may be used to improve the quality of beverages and to stabilize certain perishable products with vegetable oils [37]. In the food industry, stabilization of wine is a major application of laccase [38]. Polyphenols have an undesired effect on wine production and its organoleptic properties, making it critical to remove them from wine [38]. Many innovative treatments such as enzyme inhibitors, chelating agents and sulphate compounds have been proposed to remove phenols that cause discoloration, cloudiness and flavor changes, but have Enzymatic laccase treatment may be used as a particular mild technique to stabilize. Challenging and attractive options [18]. Since such enzymes have not yet been approved as food additives, the use of immobilized laccase can thus be classified as technical assistance, and therefore is an appropriate way to overcome such legal barriers. For example, laccase can be applied in must preparation, wine and stabilization of fruit juices [18].

**Bakery Industry 2002:** Laccase is also one of the enzymes used in the bakery industry. Laccase enzymes are added during the baking process to provide an oxidizing effect and also improve the structural strength of the dough and baked goods. Laccase imparts many properties to baked goods, such as improving crumb structure, increasing softness and volume. Low quality wheat flour can also be used in this laccase enzymatic process [39].

**Application of Laccase in the Textile Industry:** Synthetic dyes are widely used in industries such as textiles, leather, cosmetics, food and paper printing [36]. Reactive dyes are coloring molecules used to dye cellulose fibers. These dyes produce large amounts of dark waste liquid. A problem with the use of synthetic dyes is that they are resistant to biodegradation. Typically 10-50% of the initial dye load is present in the bath effluent, resulting in a darker effluent. Therefore, industrial effluents containing aromatics must be treated before their final discharge into the environment [36]. Today, environmental regulations in most countries require decolorization of wastewater prior to discharge to reduce environmental problems associated with wastewater [40]. A number of physical and chemical methods have been developed for the degradation of wastewater containing dyes [40].

Effluents from textile dyeing processes are typically treated by physical processes such as physico-chemical processes, electrokinetic coagulation, electrochemical destruction, irradiation, precipitation, ozonation, or the Katox process, which uses a mixture of activated carbon and certain gases (air). or treated by chemical processes [40]. However, due to the chemistry, molecular size, and structure of reactive dyes, these traditional processes can pose environmental challenges that can be better treated using bioprocesses [40]. Recently, enzymatic treatments have received considerable interest in decolorizing/degrading textile dyes in

wastewater as an alternative strategy to traditional chemical and physical treatments, which have significant limitations [40].

**Dye Decolorization Applications:** About 10,000 types of dyes and pigments are produced worldwide each year and are widely used in the dyeing and printing industry. Global dye production is estimated at 800,000 tonnes per year, and at least 10% of the dyes used are released into the environment as waste [41]. Most dyes are very stable to light, temperature, and microbial attack, making them untouchable. This industrial wastewater is toxic, characterized by high chemical oxygen demand (COD)/biological oxygen demand (BOD), suspended solids, and dark color. Physical and chemical processes such as adsorption, flocculation, oxidation, filtration and electrochemical processes can be used to remove color from wastewater. These methods are very expensive and have operational problems. Therefore, there is a need to develop practical biological processes for treating dye waste that can be used for a wide range of wastes [23].

Bacterial anaerobic reduction of azo dyes (which make up the majority of synthetic dyes) produces colorless dead aromatic amines, which are generally more toxic than the starting compounds. Bacterial aerobic dye degradation was restricted to chemostat-rich cultures adapted to a single dye [23]. Since wastewater contains a variety of dyes, successful decolorization of a single dye does not imply that organisms are suitable for the decolorization system. A biobleaching system must maintain high activity even after repeated exposure to different dyes. Lignin-degrading bacteria have been shown to have remarkable potential to degrade various types of dyes [14]. White rot fungi are the most efficient lignin-degrading organisms capable of degrading a wide variety of dyes, including azo dyes, heterocyclic dyes, reactive dyes and polymeric dyes [29]. Dye discoloration by white rot fungi was first reported by Glenn and Gold (1983) who developed a method to measure the ligninolytic activity of *Penicillium chrysosporium* based on the discoloration of sulfonated polymeric dyes. White rot fungi offer great advantages for breaking down stubborn compounds. The lignin-degrading enzyme produced by white rot fungi is non-substrate specific and can degrade a wide variety of stubborn compounds. Because the enzyme is extracellular, the limitation of substrate diffusion into the cell that is commonly observed in bacteria does not occur. These organisms do not need to be pretreated against specific contaminants, as enzyme secretion is dependent on nutrient limitation, nitrogen or carbon rather than on the presence of contaminants. In addition, extracellular enzymes allow white rot fungi to tolerate high pigment concentrations [36]. This paved the way for many studies on dye decolorization under conditions in which white-rot fungi produce lignin-modifying enzymes

The ability of white rot fungi to degrade laccase has been used for the oxidative detoxification or removal of various

aromatic xenobiotics and contaminants from industrial waste and contaminated soil or water [42]. Laccase catalysis can lead to direct degradation or polymerization/immobilization. Reported examples of direct degradation by laccases include dechlorination, aromatic ring cleavage, and mineralization of polycyclic aromatic hydrocarbons, bleaching of pulp or cotton mill effluents, and bleaching of textile dyes. This process involves polymerizing the contaminant itself or copolymerizing it with other non-toxic substances (such as humic substances). Polymerized contaminants are often insoluble or immobilized and can be easily removed by means such as adsorption, precipitation, or filtration [29].

**Applications in the Pharmaceutical Industry:** Laccase is used in the synthesis of various products in the pharmaceutical industry [18]. The first pharmaceutically important chemical made using the laccase enzyme was actinosine made from 4-methyl-3-hydroxyanthranilic acid. This compound has anticancer potential and works by blocking the transcription of DNA from tumor cells [43]. Another example of an anticancer drug is vinblastine, which is useful in treating leukemia. The plant Madagascar periwinkle naturally produces vinblastine. This plant produces this compound in small amounts. Catantine and vindoline are precursors of this pharmacologically important compound. These precursors are produced in large quantities and are easy to purify. Laccase is used to convert these precursors to vinblastine. Using laccase, 40% conversion of these precursors to final products was achieved. The use of laccase in such conversion reactions has enabled the production of several important compounds with useful properties such as antibiotics [44].

Catechins have antioxidant capacity, and laccase can oxidize catechins. These catechins are made up of small tannin units and are important antioxidants found in teas, herbs and vegetables. Catechins tend to scavenge free radicals, and their properties help prevent various diseases, including cancer, inflammatory and cardiovascular diseases. The antioxidant capacity of catechins is low. This property can be enhanced using laccase, resulting in the conversion of catechins into several products with enhanced antioxidant activity [45,46]. Laccase Applied in Synthesis of Hormone Derivatives Intra etc. (2005) and (Nikotra [7]) reported that laccase is capable of resolving innovative dimeric derivatives of  $\beta$ -estradiol and phytoalexin resveratrol. Isoeugenol oxidation and laccase oxidation of coniferyl alcohol or totarol, respectively, gave new dimer derivatives [46] or a mixture of dimer and tetramer derivatives. Such oxidation of substituted imidazoles produces even more complex product mixtures. These newly formed imidazoles or oligomerization products can be used for pharmacological purposes [45,46]. Aromatic and aliphatic amines can be derivatized to 3-(3,4-dihydroxyphenyl) propionic acid using laccase [47,48]. Derivatives of the above compounds have natural antiviral activity and can be used for pharmaceutical purposes [49,50].

## Conflict of Interest

No conflict of interest.

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