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A Review of Laccase Enzyme Research for Green Industry Applications

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Abstract

Laccase is an important category of multicopper oxidases that facilitate the oxidation of diverse aromatic and non-aromatic substances while simultaneously reducing molecular oxygen to water. This review investigates the potential of laccase as a sustainable biocatalyst for different bioremediation purposes. The laccase molecular structure showcases a unique configuration of copper atoms categorized into three types (T1, T2, and T3), which create the catalytic core vital for electron transfer during oxidation processes. The microbial production of laccase has attracted significant interest due to the benefits of regulated cultivation environments, the ability to manipulate genes, and greater enzyme yields compared to plant-derived sources. A variety of natural and synthetic mediators have been discovered, which broaden the applicability of laccases in industrial applications. Bacterial laccases exhibit considerable adaptability in their use for environmental purposes. In the textile sector, they aid in the breakdown of dyes and the decolorization of wastewater without producing harmful byproducts. In the pulp and paper industry, laccases provide environmentally friendly substitutes for traditional chlorine-based bleaching methods. Furthermore, these enzymes can effectively break down a range of environmental pollutants, such as phenolic compounds, polycyclic aromatic hydrocarbons, and pharmaceutical waste. Recent developments have utilized laccase in the creation of biosensors that can identify phenolic compounds, pesticides, and environmental toxins, as well as in the development of biofuel cells for renewable energy generation. This review highlights the promise of laccase as a green catalyst that supports sustainable development objectives by presenting eco-friendly alternatives to traditional chemical methods in various industrial and environmental contexts.

Keywords

Laccase, Bioremediation, Laccase-Mediator system, Decolorization, Degradation.

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INTRODUCTION

Industrial pollution is a significant issue that not only affects India but also countries around the globe, leading to numerous initiatives aimed at mitigating it. The wastewater produced by industries is a primary contributor to this pollution. Certain heavy metals present in these industrial effluents (either in their free state or adhered to suspended particles) have been identified as carcinogenic, while other chemicals, which are also present, can be toxic based on the concentration and duration of exposure. These hazardous substances are detrimental not only to human health but also pose risks to aquatic organisms [1].

The textile sector is crucial to the global economy and our everyday lives; however, it also utilizes

significant amounts of water and produces vast quantities of wastewater [2]. The primary chemical contaminants found in textile wastewater include dyes with carcinogenic amines, harmful heavy metals, pentachlorophenol, chlorine bleaching agents, halogen compounds, free formaldehyde, biocides, fire retardants, and softening agents. Due to the intricate aromatic structures and resistance to microbial breakdown of synthetic dyes, dye wastewater is inadequately decolorized by traditional biological treatment methods like the activated sludge process, and may prove toxic to the microorganisms in wastewater treatment facilities.

In recent times, the use of microorganisms that can decolourize and detoxify synthetic dyes has emerged as a promising and environmentally friendly technique for biological decolourization [3]. A survey conducted on Laccase varieties was found that they belong to the polyphenol oxidase family, which has structural and functional characteristics that are essential for removing environmental pollutants [4]. There has been an increased interest in the ability of laccase to oxidize both phenolic and non-phenolic substances in recent years. This review aims to bridge the gap by offering an extensive compilation of information on bacterial laccases documented to date. In this review, a meticulous exploration awaits, featuring on the structure of laccase enzyme, an elucidation of the enzyme's key characteristics, including mediator system, its production and a comprehensive overview of their wide-ranging applications.

Laccase (E.C. 1.10.3.2)

Laccase is a copper-containing polyphenol oxidase that was initially identified in the exudates of the Japanese lacquer tree, *Rhus vernicifera* [5], and later confirmed to be an enzyme produced by fungi [6]. The oxidation of substrates by laccase occurs through a one-electron reaction that produces a free radical, which typically undergoes further reactions through non-enzymatic pathways [7]. Laccases exhibit a remarkable lack of specificity regarding their reducing substrates, and the range of substrates oxidized varies from one laccase to another. Most laccases readily accept simple diphenols, such as hydroquinone and catechol, as substrates; however, methoxy-substituted monophenols like guaiacol and 2,6-dimethoxy phenol are frequently considered more effective. Additionally, syringaldazine is regarded as a substrate that is exclusively associated with laccase [8], [9]. These characteristics make laccase an environmentally friendly biocatalyst suitable for various industrial applications.

Origin and Properties

Laccases occur widely in fungi and less frequently in higher plants [10]. Research shows significant diversity in their molecular weights, pH optima, and substrate specificity across different sources. While fungal and bacterial laccases have been extensively studied for various applications, the potential of insect laccases remains largely unexplored [11]. Laccase enzymes have been extensively studied in fungi, including Ascomycetes, Basidiomycetes, and Deuteromycetes, as highlighted by Brijwani *et al*. [12]. The first bacterial laccase was characterized in *Azospirillum lipoferum* in 2000 by Diamantidis *et al*., marking a significant milestone [13]. However, research on bacterial laccases remained limited until the last two decades, when their unique properties garnered interest.

Unlike fungal laccases, bacterial laccases exhibit superior thermostability, optimal activity in alkaline pH conditions, and tolerance to saline environments. These traits make bacterial laccases more suitable for various industrial applications as they can withstand high temperatures and pH levels [4], [14]. The practical use of fungal laccases is constrained by challenges such as their filamentous mycelium structure and slow growth rates, which complicate downstream processing and increase costs. These limitations reduce their feasibility for large-scale industrial applications [15]. Conversely, bacterial laccases offer a promising alternative due to their stability and adaptability in extreme conditions.

Structure and Mechanism of laccase

The three-dimensional architecture of laccases, characterized by multiple copper atoms coordinated within the enzyme's active site, plays a pivotal role in facilitating their oxidative property. The unique arrangement of these copper centres imparts laccases with the ability to catalyse a diverse array of substrates, ranging from phenolic compounds to recalcitrant pollutants. Moreover, the structural diversity observed among laccase isoforms further enhances their adaptability and catalytic efficiency across various environmental conditions and industrial applications. In short, a deeper exploration of the structural peculiarities of laccase enzymes not only enriches our understanding of their catalytic mechanisms but also paves the way for unlocking their full potential in addressing contemporary challenges in bioremediation, biocatalysis, and beyond.

Laccase enzymes (EC 1.10.3.2) function through a sophisticated redox mechanism involving four copper atoms arranged in three distinct centres: Type 1 (T1), Type 2 (T2), and Type 3 (T3) [16]. The catalytic cycle begins when a substrate, typically a

phenolic compound, undergoes one-electron oxidation at the T1 copper site, generating a free radical intermediate while reducing Cu^{2+} to Cu^{+} [17]. This electron is subsequently transferred through an internal pathway to the trinuclear T2/T3 copper cluster, where molecular oxygen binds and is eventually reduced to water after receiving four electrons from four substrate molecules [18].

The reaction can be represented as **4 substrate-H + O_2 → 4 substrate• + 2H_2O [19].**

This mechanism allows laccases to oxidize a diverse range of substrates including phenols, polyphenols, aromatic amines, and certain inorganic compounds, with the resulting radical products often undergoing further non-enzymatic coupling, fragmentation, or polymerization reactions [20]. The broad substrate specificity and oxygen-dependent catalysis make laccases particularly valuable in various industrial and environmental applications, from lignin degradation to xenobiotic compound detoxification [21].

Microbial production

Several literature reports reveal that laccase can be produced by selection of suitable substrate, different production medium configurations under different climatic conditions [22], [23]. Production of laccase is generally affected by various fermentation factors; such as various carbon and nitrogen sources. Among the different carbon sources, glucose was found as best carbon source in laccase production and shows maximum activity of 124 U/mL, slightly add 2% starch in media and the highest enzyme activity observed was 288 U/mL [24].

Economically cheaper and commonly available agricultural wastes residues are the excellent sources. The utilization of lignocellulosic agro-industrial residues like; rubberwood dust, rice straw, empty fruit bunch, and sugarcane bagasse were seen by many researchers in laccase production by fungus in a growth medium with 1% (w/v) substrate concentration.

The laccase-mediator system.

Represents a significant advancement in expanding the catalytic property of laccase enzymes by incorporating small molecular weight compounds called mediators that act as electron shuttles between the enzyme and recalcitrant substrates [25].While native laccases are restricted to oxidizing compounds with relatively low redox potentials, mediators such as ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), HBT (1-hydroxybenzotriazole), and TEMPO (2, 2, 6, 6-tetramethylpiperidine-1-oxyl) can be oxidized by laccase and subsequently diffuse away to oxidize non-phenolic substrates that would otherwise be inaccessible to the enzyme's active site [18].

The efficiency of substrate oxidation by a laccase depends on the difference between the redox potentials of the substrate and the pocket size of site T1 [26], [15]. So, some low-molecular weight redox mediators are used to expand the laccase substrate range especially for substrates with higher redox potentials. [27], [28], [29]. Commonly used laccase mediators include synthetic mediators such as 2,2'-azino-bis (3-ethylbenzothiazoline-6sulfonate) (ABTS) and 1-hydroxybenzotriazole (HBT) as well as natural phenolic mediators such as acetosyringone (ACS) and syringaldehyde (SA) [18], [28].

This two-step mechanism involves initial oxidation of the mediator by laccase at the T1 copper site, followed by mediator-facilitated oxidation of the target substrate through various mechanisms including hydrogen atom transfer (HAT), electron transfer (ET), or ionic mechanisms [30]. The LMS has dramatically extended laccase applications in various industrial processes including pulp delignification, textile dye decolorization, bioremediation of recalcitrant pollutants, and organic synthesis, enabling reactions with non-phenolic lignin components, synthetic dyes, polycyclic aromatic hydrocarbons, and other complex compounds that resist direct laccase oxidation [31].

Purification and biochemical properties of laccases

Laccases are mostly intracellular or present in periplasmic space but recently extracellular laccases were identified [4], [32]. The easy extraction, purification and higher production of extracellular laccases make them more preferable for industrial applications [14], [32]. Purification is an important step in the characterization of different laccases from different sources which also varies from species to species. Most commonly followed concentration methods are ammonium sulphate precipitation or acetone precipitation. The purification steps are ion exchange chromatography or gel filtration which is sometimes followed by ultrafiltration [15]. By acetone precipitation and anion exchange chromatography an extracellular laccase was isolated and purified from *Pseudomonas* sp. LBC1 [33].

The catalytic performance of laccases can be described by their activity and stability in different pH and temperature conditions. With phenolic substrates the pH activity profiles of laccases are often bell shaped with optima around 4-6. [9], [34]. The decrease in laccase activity in neutral or alkaline pH values is affected by increasing hydroxide anion inhibition. In contrast to their activity, stability of laccases is generally highest at pH values around 8-9

[35], [36], [9]. Temperature stabilities of laccases vary considerably, depending on the source organism. In general, laccases are stable at 30-50°C and rapidly lose activity at temperatures above 60°C [37], [38], [39]. The most thermostable laccases have been isolated from bacteria. The half-life of *Streptomyces lavendulae* laccase was 100 minutes at 70°C and that of *Bacillus subtilis* CoA was 112 minutes at 80°C.

APPLICATION OF BACTERIAL LACCASE:

Laccases are becoming increasingly popular due to their ability to degrade environmental pollutants, domestic waste materials, pesticides, dyes, pharmaceutical and cosmetic products, and diverse chemicals from agriculture and industry [40], [41]. Also, laccase has become a potent industrially relevant enzyme due to its wide range of applications such as delignification of lignocellulosic material, in food industries [42], [43], pulp and paper industry [44], [45], and also used in the development of biosensors [46], [47].

Some of the important industrial applications have been discussed below:

Role of Laccase in textile dye degradation & decolorization:

Artificial paints [48] and dyes used in textiles, food, pharmaceuticals, paper, and chemicals industries [49] are some of the most prevalent environmental hazards in the modern world. Laccases have gained significant importance in recent years for dye decolorization. These have the ability to oxidize both phenolic and nonphenolic chemicals, making them effective in decolorizing dyes [50]. Laccases from *Bacillus safensis* DSKK5 have been shown to successfully decolorize widely used textile dyes [51]. Similarly, laccases derived from *Geobacillus* sp. JS12 have demonstrated effective decolorization of textile dyes such as Congo red and Malachite green [52]. Among the environmental factors, the temperature is a very important factor that affects the functioning of both intracellular and extracellular microbial enzymes [53]. The efficiency of enzymatic degradation potentially depends upon the availability, activity, and adaptation of the chosen microorganism. Laccase show their potential in promoting eco-friendly and sustainable textile production by the effective degradation and decolorization of different textile dyes.

Role of laccase in pulp and paper industry:

The paper manufacturing and recycling industries encounter significant challenges in eliminating phenolic compound lignin. This task can be accomplished using various chemical methods; however, these methods are often hazardous and contribute to environmental pollution. This has led to the adoption of ligninolytic and hemicellulolytic enzymes, which meet all necessary requirements. Enzymatic deinking enhances brightness, whiteness, and reduces remaining ink, ultimately conserving energy and lowering overall costs [54], [55]. Individual enzymes can effectively break down phenolic compounds due to their elevated redox potential. The addition of mediators enhances both the accessibility and capacity of the enzyme against nonaromatic ring-containing substances [56]. Investigated the use of laccase derived from the proteobacterium JB for bio bleaching soda pulp employing a statistical approach. Sondhi *et al*. [14] examined the use of an extracellular thermo-alkali stable laccase from *Bacillus tequilensis* SN4 for the bio bleaching of pulp. The combination of enzymes, specifically xylanase and laccase, proves to be an efficient method for reducing the lignin content and related compounds in pulp [57]. The partnership of xylanase and laccase exhibited a synergistic effect that improved the properties of the pulp. Dual cultivation presents several benefits, such as better substrate utilization, increased enzyme yields, and a suppressive effect on the growth of undesirable microorganisms.

Role of laccase in pollutant degradation

The primary pollutant that is uniformly dispersed across a natural environment, such as soil, air, or water, is polycyclic aromatic hydrocarbons, or PAHs. An angular, linear, or clustered benzene ring makes up their structure [58],[59]. The majority of these toxins and their byproducts are carcinogenic to living things and dangerous to people. Because of their sluggish rate of degradation and limited water solubility, these aromatic hydrocarbons are xenobiotics [60]. There are currently few reports that demonstrate how bacteria can break down xenobiotic substances [58], [61]. The laccase enzyme is thought to be responsible for converting polycyclic aromatic hydrocarbons to their quinines and then carbon dioxide. According to Rajeshwari and Bhuvaneswari [62], 92% of bisphenol A (BPA) is converted into 4-ethyl-2-methoxy phenol by isolated laccase from *Bacillus* sp. PK4. identified by Menaka *et al*. [61]. One of the most promising isolates having the ability to degrade 2, 4-dichlorophenol was identified as *Bacillus subtilis.* A low-cost, eco-friendly, and incredibly effective biochemical method is laccase-mediated chlorophenol elimination.

Development of biosensors and biofuel Cells

Laccase is widely studied for their use in formation of biosensor and biofuel cell [63], [64]. The broad substrate range of laccase makes it capable to detect

various phenolic compound and so can be useful in biosensor technology [65], [66]. Biosensor composed of laccase-based electrode are used for detection of phenolic components in wine, catechols in tea [65]and lignin and phenols in wastewater. The laccase-based biosensors are used for medical applications [68]. Not only in biosensor, laccase is also applicable in formation of fuel cell [69]. The major reason for this interest is the use of oxygen as a substrate, which is converted into water. A zinc-laccase biofuel cell was developed by Slomczynski *et al*. [70] which is operated under open ambient conditions.

CONCLUSIONS AND FUTURE PROSPECTS

This review article encompasses the role of laccases as a green solution to the biodegradation of environmental contaminants and as biocatalysts. Laccase is one of the earliest enzymes to be identified in 1883, but determining its activity is still a scientific difficulty and a barrier to its full potential as a biocatalyst. An oxidase belonging to the blue multi-copper oxidases family, laccase has a broad range of substrates, such as aromatic amines, substituted phenols, and substances associated to lignin. Its one-electron method produces water as a reaction product and just needs oxygen. Laccase's properties make it a biocatalyst of interest in a wide range of applications, including the food, pharmaceutical, textile, pulp and paper, and biorefinery sectors. However, for laccase to be used on an industrial scale, its activity needs to be able to be consistently measured in complicated matrices and on complex substrates. This paper seeks to outline new and existing techniques to place them in the context of a potential industrial enzyme.

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