KAVAKA 61(4): 72-86 (2025) DOI: 10.36460/Kavaka/61/4/2025/72-86

Laccase Enzyme: A Mushroom Derived Secondary Metabolite

Rajni Gupta[1]*, Promila Gupta[2], Sunil Kumar Dhiman[1], Ram Babu[1], Shivesh Raj Singh[1], and Saksham Chauhan[1]

[1]*Department of Botany, Kirori Mal College, University of Delhi, Delhi – 110 007, India.*
[2]*Department of Biotechnology, I.P. University, Delhi – 110 007, India.*
**Corresponding author Email: rgupta@kmc.du.ac.in*

(Submitted on July 23, 2025; Accepted on August 28, 2025)

ABSTRACT

Mushrooms are essentially used in many aspects due to the various properties of their metabolites as multiple secondary metabolites are produced by them. Laccase enzyme being one of them generally used for degrading lignin and in bioremediation. Laccase is an oxidizing enzyme, that produces free radicals. The textile, pulp, and paper industries release a lot of waste into the environment and the disposal is a big problem. Studies have been carried out to find a solution eliminating these waste products for recreating the environment from Laccase enzyme. Laccase uses oxygen and produces water as a by-product. Laccase can degrade many compounds, both phenolic and non-phenolic. Their ability to act on a variety of substrates and remove a variety of contaminants makes them useful for many purposes in a variety of industries, including the paper, pulp, textile, and petrochemical industries. Present review discusses various secondary metabolites produced by mushrooms and focus on the Laccase enzyme and its Industrial uses.

Keywords: Bioremediation, Environment, Industrial uses, Laccase, Mushrooms

INTRODUCTION

The mushrooms are neither plants nor animals. They are a distinct type of organism having a mycelium at the base which is a web-like structure and fruiting body known as basidiocarp, varies in size from smaller to larger, and various colours of mushroom can be seen in nature. The hyphae absorb nutrients from the environment, they can be epigeal and hypogeal in formation. Although they don't have chlorophyll, some people still consider them plants. Based on cell wall composition, nutrition, and ability to photosynthesize they are also not considered animals. Mushrooms are unique, they are capable of forming symbiotic relationships with other plants, animals, and even other fungi. The fungi provide the host organism with essential nutrients while the fungus receives carbohydrates in return.

Mushrooms are exploited because of their nutritional, pharmaceutical, economic, and environmentally friendly nature (Rahi and Malik 2016; Vetter 2019; Niazi and Ghafoor 2021; Gupta and Singh 2024). They are considered superfoods, with low calories, fat and are a great source of vitamins and minerals. Many dishes can be made out of them, alternate of meat. Furthermore, mushrooms are an excellent dietary fiber source, rich in amino acids i.e., niacin, leucine, valine, and iso leucine etc. These amino acids are absent in animals. They have 2-4 times more vitamins than vegetables and 12 times more vitamins than fruits. Mushrooms have more proteins than legumes (**Figure 1**).

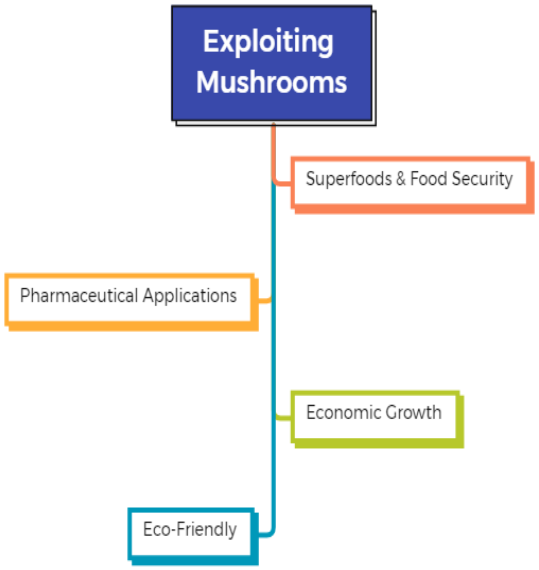

Figure 1: The various purposes of exploiting the mushrooms

There are various types of mushrooms but broadly they can be classified as edible and non-edible mushrooms." In the last 56 years, the consumption of mushrooms has increased 21-fold." Despite being highly marketable these days they are still consumed directly from the wild. From many years, the question about their edibility remains

unresolved. There is no common consensus among researchers about the edibility status of mushrooms because much of the knowledge comes from anecdotal evidence and traditional know-how of eating them. There are often frequent variations across publications increasing the debate even further. The different tribal and local communities had developed this habit of checking the edibility of wild mushrooms based on their adventurous and risk-taking nature before consuming them. Their risk-taking nature can be attributed to their cultural bias and attitude towards their way of consuming mushrooms. A system was developed to categorize and assign edibility status to 2,786 mushroom species from 99 countries, analyzing 9,783 case reports. There are 2,189 edible species, of which 2,006 are safe to eat. Additional 183 species require pre-treatment or may cause allergic reactions. Moreover, 471 species had uncertain edibility due to insufficient or incomplete consumption evidence, and 76 species had unresolved edibility and toxicity. Asia is home to the most edible mushrooms in the world, followed by Europe**.** *Russula* is regarded as one of the largest edible mushroom genera. (Li *et al.,* 2021).

Mushrooms are remarkable not only for their nutritional value but also for their edibility. Moreover, they are a good source of various secondary metabolites, including enzymes, which have diversified applications.

SECONDARY METABOLITES OF MUSHROOMS

Secondary metabolites in fungi are compounds that, unlike primary metabolites (proteins, carbohydrates, nucleic acids, and lipids), are not essential for immediate fungal growth. These metabolites are produced during the stationary phase of growth, a period after the initial rapid growth phase when the fungus is not actively dividing or increasing in biomass. While not essential for survival, secondary metabolites play a crucial role in a fungus's defense, competition, and communication within its environment. Secondary metabolites with important medical and agricultural applications include antibiotics like penicillin, cholesterol-lowering agents like lovastatin and mevastatin, immunosuppressing drugs like cyclosporin A, and plant hormones like gibberellic acid. Enzymes are also a part of secondary metabolite and they can break down complex substrates in their surroundings into nutrients with effective absorbion (Chaudhary *et al.*, 2022). Industrially relevant fungal enzymes include amylase from *Aspergillus niger* and *Rhizopus oryzae* for starch processing, phytase from *Aspergillus niger* for phosphate release, and the hepatitis B vaccine surface antigen produced by *Saccharomyces cerevisiae*. However, the specific relationship between these enzymes and secondary metabolites remains unclear.

Enzymes are proteins that act as catalysts to chemical reactions in living organisms. There has been tremendous potential of mushroom-derived enzymes for various industrial applications, including food processing, textile production, and paper manufacturing. Cellulases and xylanases are crucial for breaking down plant cell walls, making them valuable in biofuel production and paper recycling. Similarly, laccases are used in textile industries for dye decolorization and denim bleaching. The sources emphasize the advantages of fungal enzymes over those from other sources, citing their high yield, cost-effective production using inexpensive media, and the remarkable catalytic versatility of fungal cells. Hatimuria *et al.,* (2023) discussed about the laccase activity and stability in various deep eutectic solvents and their correlation between laccase interaction with different types of DES, components and description which can regulate the activity of laccase (**Table 1**).

Table 1: Functional Diversity of Fungal Enzymes and Their Industrial Uses

S. No.	Enzyme	Source	Function
1	Laccase	*Pleurotus species*, *Tuber maculatum*	Lignin degradation
2	Manganese peroxidase	*Pleurotus species*	Lignin degradation
3	Lignin peroxidase	*Pleurotus species*	Lignin degradation

S. No.	Enzyme	Source	Function
4	Cellulase	*Pleurotus species, Tuber maculatum*, *Lentinula edodes*,	Breaks down cellulose into glucose; commercial applications in food, fermentation, textile, and paper industries
5	Xylanase	*Pleurotus species*, *Tuber maculatum*, *Tuber aestivum*,	Breaks down hemicellulose; commercial applications in food, fermentation, textile, and paper industries
6	Amylase	*Pleurotus djamor* var. *Roseus*, *Pleurotus* species *Tuber maculatum*, *Tuber aestivum*, *Lentinula boryana*,	Breaks down starch into glucose
7	Protease	*Pleurotus species, Tuber maculatum*	Breaks down proteins
8	Lipase	*Pleurotus sp., Tuber maculatum*	Breaks down fats
9	Peroxidase	*Pleurotus species, Tuber maculatum*, *Tuber aestivum*	Catalyzes oxidation reactions
10	Catalase	*Tuber maculatum*, *Tuber aestivum*	Catalyzes the decomposition of hydrogen peroxide to water and oxygen
11	Tannase	*Pleurotus species*	Degradation of tannins

Sources: Luz *et al.,* 2012; Polizeli and Rai 2013; An *et al.,* 2016; Rahi and Malik 2016; Elkhateeb *et al.,* 2022

Laccases are also essential to the carbon cycle because they aid in the subsequent breakdown of organic materials by dissolving lignin into smaller molecules. In addition, laccase enzymes from mushrooms have important biotechnological uses in industries, including food processing, paper, pulp bleaching, as well as bioremediation, where they aid in the breakdown of environmental contaminants (Sharma *et al.*, 2017). Among all the enzymes produced by mushrooms, laccase is an enzyme that is currently a matter of interest due to its various applications and increased use. Therefore, after understanding more about the laccase enzyme and the relationship of mushrooms with it, we can maintain environmental health and also assist in numerous industrial processes.

WHAT IS LACCASE ENZYME?

Laccase belong to the group of phenol oxidases; they are very common, widely dispersed oxidative enzymes produced by a variety of fungus and found in many plants. Laccase was first described by Yoshida in 1883 from the secretion of the Japanese lacquer tree *Rhus vernicifera*. However, in 1896, it was discovered that laccase was a fungal enzyme (Yoshida, 1883; Thurston, 1994) Laccases are copper based enzymes that catalyze the oxidation of various organic and inorganic substrates, such as monophenols, diphenols, and polyphenols, aminophenols, methoxy phenols, aromatic amines and ascorbic acid, by the four-electron reduction of oxygen to water (https://pubmed.ncbi.nlm.nih.gov/12101303/). Laccases utilizes molecular oxygen for the catalytic activity (Bassanini *et al.,* 2021). They have been released by the majority of lignin-degrading basidiomycetes which has been reported as necessary for lignin breakdown in a fungus missing, peroxidases (Kahraman and Gurdal 2002, Eggert *et al.,* 1997). Additionally, they can detoxify a variety of environmental toxins and are capable of catalyzing the oxidation of a large variety of aromatic amines and phenolic compounds due to this they are the subject of extensive research but in the presence of low molecular mass molecules function as mediators and its substrate range has been expanded to include non-phenolic compounds (Bourbonnais and Paice 1990; Eggert *et al.,* 1996). The activity of laccase or purified isoenzymes is determined by photometric assays using phenolic substrates and monitoring colour oxidation products. These fungal laccases have a wide range of uses in the field of biotechnology; these include the detoxification and removal of the colour of

sewage, pulp bleaching, the elimination of phenol compounds from wines, organic synthesis, biosensors, the formation of complex medical compounds, and the blocking of dye transfer in detergents and washing powders. Many of their uses have been patented (Yaver *et al.,* 2001). Laccase enzyme have various catalytical activity and higher yields (Chan *et al.,* 2021).

Occurrence of laccase

Most of the species of higher plants and fungi consist of laccase, as well as they are present in bacteria (Benfield et. al. 1964; Diamantidis *et al.,* 2000). Laccase was classified under blue oxidase in the Enzyme Commission (EC) nomenclature, therefore they are also called oxidative enzymes due to their property of oxidizing many phenolic and non-phenolic compounds, including aromatics, diphenols and aliphatic amines using molecular oxygen as the sole electron acceptor (Surwase *et al.,* 2016). Laccase has been found in cabbages, radishes, beets, apples, asparagus, potatoes, pears, and many vegetables (Levine, 1965). Additionally, it is discovered that several other insect genera contain laccase, including *Diploptera, Drosophila, Bombax, Manduca, Rhodnius, Papilio, Musca, Oryctes, Tenebrio, Lucilia, Sarcophaga, Schistocerca,* and *Phormia* (Xu, 1999). Most of the laccases has been reported from higher fungi like ascomycetes, basidiomycetes, and deuteromycetes fungi (Assavanig *et al.,* 1992). The laccase from *Monocillium indicum* was the first and one of the laccases to be identified with peroxidative activity (Thakker *et al.,* 1992). Also, the studies on the breakdown of lignin in fungi have been focused on white-rot basidiomycetes, rather than ascomycetes and deuteromycetes. The most common laccase producers are the wood-rotting fungi *Trametes hirsuta*, *Trametes versicolor*, *Trametes villosa, Trametes ochracea, Pleurotus eryngii, Lentinus tigrinus, Coriolposis polyzona, Cerena maxima,* etc. (Morozova *et al.,* 2007) but few bacteria have also been found to have laccase activity, including *Bacillus subtilis*, *Marinomonas mediterranea, Azospirillum lipoferum* and *Streptomyces griseus.*

Laccase producing mushroom species

The production of laccases can be influenced by factors such as the mushroom species, growth substrate, and cultivation conditions. *Pleurotus ostreatus* produces laccases cultivated on different substrates, such as coffee husks, sawdust, and eucalyptus bark, with variations in enzyme activity observed depending on the substrate composition and the addition of supplements like rice bran (Luz *et al.,* 2012; Polizeli *et al.,* 2013; Elkhateeb *et al.,* 2022)

Laccases are produced by mushrooms such as those in the *Flammulina* genus, a group of medicinal and edible mushrooms cultivated and consumed in East Asia. Like typical white-rot fungi, *Flammulina* species are highly adaptable to growing on various agroindustrial lignocellulosic wastes because of their ability to synthesize hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) extracellular enzymes (An *et al.,* 2016) (**Table 2**, **Figure 2**)

Table 2: Overview of Laccase Production across different Mushrooms

S. No.	Mushroom Species (Sources)	Laccase enzyme production
1	*Pleurotus eryngii*	Produce laccase and cellulase enzymes when grown on substrates such as kenaf stalks, bulrush stalks, and cottonseed hulls.
2	*Pleurotus florida*	Produces laccase.
3	*Pleurotus giganticus*	A wild, edible mushroom was identified as a laccase producer when grown with 0.02% guaiacol as the substrate.
4	*Pleurotus ostreatus*	Produces laccase and noted for its nutritional and medicinal characteristics, depend on its growth substrate, including agricultural wastes. *Pleurotus ostreatus* laccase, in the presence of natural phenolic compounds, enhanced the transformation of malachite green.
5	*Pleurotus ostreato roseus*	Produces protease. when this mushroom cultivated on *Dioscorea alata* produced high protease titers.

S. No.	Mushroom Species (Sources)	Laccase enzyme production
6	*Pleurotus pulmonarius*	Production of laccase isoforms by this mushroom is influenced by phenolic and aromatic compounds. One study found copper improved laccase production in *Pleurotus pulmonarius.*
7	*Pleurotus sajor-caju*	Produces laccase and protease. Novel alkaline and detergent stable protease (SPPS) from the CTM10057strain of this mushroom
8	*Pleurotus sapidus*	Produces laccase
9	*Agaricus bisporus*	Produces laccase from two laccase genes, *lcc1* and *lcc2*.
10	*Agrocybe aegerita*	Produces laccase, particularly in an extracellular medium containing copper sulfate, Tween 80, and 4,6-dimethyl-2-mercaptopyrimidine.
11	*Ganoderma lucidum*	Produces laccase. Cultivated from *Ganoderma lucidum* on cotton waste to produce multiple forms of laccase in a submerged culture. Another study found that *Ganoderma lucidum* laccase, in the presence of natural phenolic compounds, enhanced the transformation of malachite green.
12	*Hericium erinaceus*	Produces laccase when cultivated for laccase production in a malt extract and peptone growth medium.
13	*Hypsizygus marmoreus*	Produces laccase, particularly in an extracellular medium containing copper sulfate, Tween 80, and 4,6-dimethyl-2-mercaptopyrimidine.
14	*Lentinula edodes*	Produces laccase. commercial strains of *Lentinula edodes* grown on coffee beans produced cellulase enzymes.
15	*Lentinus sajor-caju*	A wild, edible mushroom was identified as a laccase producer when grown with 0.02% guaiacol as the substrate.
16	*Schizophyllum commune*	Produces laccase. High enzyme production on the hardwood sawdust
17	*Termitomyces clypeatus*	Produces protease. Proteases from this mushroom have been found to have antiproliferative activity on human hepatoma hepg2 cells.
18	*Trametes modesta*	Produces laccase. One study used *Trametes modesta* laccase for the decolorization of textile dyes.
19	*Trametes pubescens*	Produces laccase and phytase.
20	*Trametes versicolor*	Produces laccase. One study achieved a yield of up to 1000 mg L−1 of *Trametes versicolor* laccase expressed in *Trichoderma reesei.*
21	*Trametes villosa*	Produces laccase. One study purified, characterized, molecularly cloned, and expressed two laccase genes from *Trametes villosa.*
22	*Volvariella volvacea*	Produces laccase. One study found that the strain *Volvariella volvacea* grown on cotton waste produced multiple forms of laccase in a submerged culture.

*Source: Wood, 1980 ; Sivakumar *et al.,* 2010 ; Ravikumar *et al.,* 2012 ; Luz *et al.,* 2012 ; Polizeli and Rai 2013 ; El-Batal *et al.,* 2015 ; An *et al.,* 2016; Xu *et al.,* 2020; Elkhateeb *et al.,* 2022b; Kang *et al.,* 2024

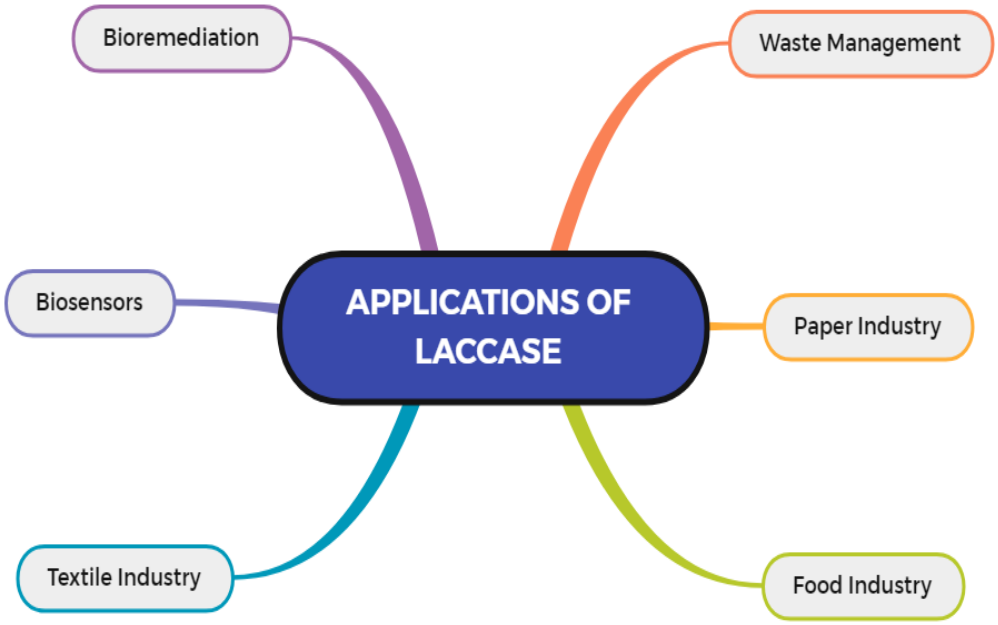

Figure 2: Mind-Map showing applications of laccase

MOLECULAR PROPERTIES OF LACCASE ENZYME

Laccase is a glycosylated monomeric or homodimeric protein in bacteria and viruses that generally contains comparatively less sugar (10-25%) than in plant enzymes. Carbohydrate compounds in laccase include simple sugars such as galactose, glucose, mannose, hexosamine, arabinose, and fucose. (Rogalski and Leonowicz 2004). On SDS-PAGE, most laccases show mobility at a molecular weight of 60–100 kda, of which 10–50% is attributable to glycosylation. Mannose is one of the main components of laccase-dependent carbohydrates. The isoelectric point (pH) of fungal laccases is between 3 and 7, plant laccases between 9 and 5.2, and the optimal pH value of laccase is between 3.6 and 5.2. Fungal enzymes have a lower optimal pH, probably because they are well suited to growth in acidic conditions, whereas the optimal pH of intracellular plant laccases is close to the physiological range. Therefore, the difference in optimal pH may be due to the difference in body activity **(Figure 3**). Purified laccase exhibits a characteristic blue appearance with electron absorption around 600 nm. A typical UV-visible laccase spectrum (resting state) shows two maxima around 280 and 600 nm and a shoulder around 330 nm. The absorbance ratio between 280 nm and 600 nm is generally 14 ~ 30, and the absorbance ratio between 330 nm and 600 nm is 0.5 ~ 2. (Xu, 1999). Type I copper (T1) gives the enzyme a dark blue color, provides strong absorption near 600 nm and can be detected by EPR. Type II copper (T2) is colorless but has EPR detection; type 3 copper (T3) has a pair of copper atoms and weak absorbance near the UV spectrum but no EPR light red. The second type of copper is easy to remove and can usually be done during cleaning. Type 2 copper can be regenerated under aerobic and anaerobic conditions. Studies on laccase derivatives have shown that type 2 copper is bonded to three nitrogen atoms. Also, type 2 copper has been shown to play an important role in the structural instability of the copper 3 active site anionic bond. Type I copper centers without a methionine ligand have also been found to be unstable. This is true for all fungal infections (Pointek *et al.*, 2002).

Although most laccases fit this description, some highly purified laccases do not exhibit these properties. The results showed that the laccase isolated from the fungal culture was yellow-brown, had no blue oxidase spectrum, and also showed a typical EPR spectrum. The active center of the yellow enzyme appears to change the oxidation state of copper, possibly due to the addition of aromatic lignin degradation products. Interestingly, there is evidence that yellow laccase can directly oxidize the non-phenolic lignin structure and veratrol in the presence of oxygen but without a diffusion medium (Rogalski and Leonowicz 2004).

Laccase enzyme consists majorly 3 copper sites that not only impart colours but also have other molecular properties.

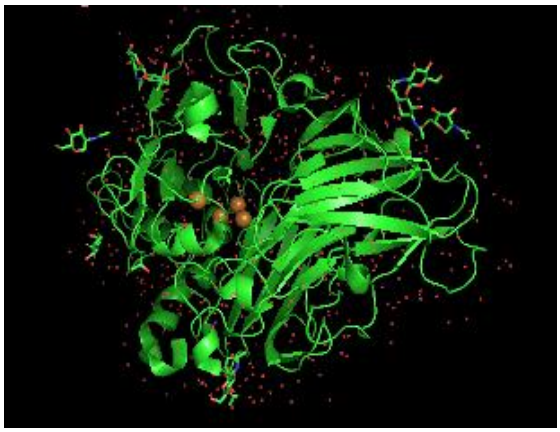

Figure 3: Demonstrating a Crystal Structure of a Laccase from the Fungus *Trametes versicolor* at 1.90-A Resolution Containing a Full Complement of Coppers.

LACCASE AS A DETOXIFYING AGENT

Laccase acts as a detoxifying agent by purifying the environment from toxic substances as they cause aryl-alkyl cleavage, Cα-Cβ cleavage, and Cα oxidation by attacking the phenolic subunits of lignin. Laccase catalysis is thought to include the following steps: site 1 is the Reduction of type 1 copper through substrate reduction, followed by step 2, which is Internal electron transport between copper types 1, 2, and 3. the final step is the reduction of oxygen to water at copper sites type 2 and type 3. Laccases under oxidation reduce substrates, they often produce free radicals (cations) and lose an electron in the process. These free radicals are often unstable and can go through non-enzymatic processes like hydration, disproportionation, or polymerization, or they can go through further oxidation that is catalyzed by laccase, like the creation of quinone from phenol (Xu, 1999). Also, the redox conditions formed due to laccase activity can regulate the electron transport from the substrate to type 1 copper. This detoxifying property of laccase also has an important role in pollution control and bioremediation in the environment.

LACCASE AS A BIOREMEDIATION AGENT

Fungal species can flourish in extreme environments primarily because they produce unique enzymes that are capable of executing intricate chemical reactions (Viswanath *et al.,* 2008 ; Shraddha *et al.,* 2011). Laccases, which are vital blue multicopper oxidases, can oxidize various substrates and convert oxygen into water. There has been increasing interest in laccases recently due to their potential to detoxify pollutants and bioremediate phenolic compounds (Bollag *et al.,* 1998; Singh *et al.,* 2009; Singh *et al.,* 2011). These enzymes can decompose materials such as wood, plastic, paint, and jet fuel into nutrients. Some of these enzymes are already utilized in the pulp and paper industry and the synthesis of fine chemicals (Viswanath *et al.,* 2008). Laccases also play a crucial role in the breakdown of lignin and phenolic compounds, and this function has been assessed in numerous biotechnological applications, including the degradation of dyes and their ability to use only molecular oxygen for catalysis makes them ideal for biotechnological applications in transforming or immobilizing xenobiotic compounds (Rodriguez *et al.,* 2006). Laccases are widely occurring enzymes in nature, but the physiological roles of these biocatalysts, whether secreted or intracellular, vary among different organisms, yet they all facilitate polymerization or depolymerization processes (Riva, 2006). Furthermore, recent studies suggest that lignin-degrading fungi, also known as white-rot fungi-which cause wood to appear bleached—such as *Phanerochaete chrysosporium* and *Trametes versicolor*, might be able to replace some of the chemical processes currently used. Some of the examples of laccase helping in bioremediation of various Xenobiotics chemical compounds (**Table 3).**

Table 3: Different fungal species containing Laccase enzyme for specific roles in Bioremediation

S. No.	Fungal Species	Role in Bioremediation	References
1.	*Trametes sp.*	Degradation of phenolics	Michizoe *et al.,* 2005
2.	*Pleurotus eryngii*, *P. Ostreatus*, *P. Pulmonarius* and *P. sajor caju*	Degradation of 2,4-dichorophenol and benzo (a) pyrene	Rodriguez *et al.,* 2004

S. No.	Fungal Species	Role in Bioremediation	References
3.	*Ganoderma sp.* WR-1	Decolouration of an industrial effluent containing a mixture of dyes	Revankar and Lele 2007
4.	*Trametes pubescens*	Decolouration of synthetic dyes	Osma *et al.,* 2007
5.	*Cariolopsis polyzona*	Degradation of NP, BPA and TCS	Cabana *et al.,*2007
6.	*Ischnoderma resinosum*	Decolouration of textiles dyes	Kokol *et al.,* 2007
7.	*Cyanthus bulleri*	Decolouration of textile dyes	Mishra and Bisaria 2006
8.	*Trametes versicolor*	Decolouration of textile dye	Blanquez *et al.,* 2007

LACCASE IN POLLUTION MANAGEMENT

Pollution from man-made chemicals is a big environmental issue. These chemicals, unlike natural ones which break down easily, are very hard for microbes to get rid of. Because of stricter environmental rules, scientists are looking for new ways to clean up pollution that are better for the environment. One promising area of research is using enzymes to break down pollutants. Laccase enzymes are especially good for this because they can break down a wide variety of pollutants, they don't need any extra chemicals to work, and they are easy to collect and use because they are found outside the cells of fungi also, they are pretty tough and can survive in the environment. Fungi can be easily grown to produce more of these enzymes. Laccase helps in the detection of various pollutants in the environment, like phenols, lignin, or xenobiotics. The phenols are by products of the decomposition of organic natural components such as tannins, lignins, and humic chemicals because of their highly poisonous, carcinogenic, and allergic properties, some phenols and related aromatic chemicals must be identified and removed from the environment. Determining the toxicities of the majority of phenols is crucial for assessing the overall toxicity of an environmental sample because they exhibit varying degrees of toxicity. Chromatographic separation is typically performed on phenolic compound detection. However, the process of separation is laborious and frequently necessitates prior focus. Furthermore, the apparatus is costly and typically non-portable. It will be beneficial to possess a tool that makes it possible to identify phenols in aqueous solutions at low micromolar concentrations with little sample preparation. (Vianello *et al.,* 2004). The task is hence difficult to detect and monitor phenols in wastewater because the maximum amount permitted by the European Community in wastewater is less than 1 mg/L in European Community countries (European Community "Urban Water Directive" 91/271/EC), 1 mg/L in Japan (Water Pollution Control Law and Tochigi Prefecture ordinances), 0.1 mg/L in Brazil (Secretaria de Saúde e do Medio Ambiente, State Law 5/89), and 0.5 mg/L in the US (Ersöz *et al.,* 2003) but due to property of laccase as a biosensor helps us to identify and remove pollutants easily (**Table 4).**

Table 4: Laccase as a bioindicator in different species for identifying toxic substances

S. No.	Fungal Species	Role in the detection of toxic substances	References
1.	*Rigidoporus lignosus*	Biosensor for the detection of phenols in OMW	Vianello *et al.,* 2006
2.	*Trametes versicolor*	Biosensor for the determination of phenols in environmental control	Roy *et al.,* 2005
3.	*T. versicolor, Aspergillus niger* and *Agaricus biosporus*	Biosensor for the determination of xenobiotics in wastewater	Timur *et al.,* 2004

S. No.	Fungal Species	Role in the detection of toxic substances	References
4.	*Trametes hirsuta*	Biosensor for monitoring lignin in wastewater from pulp and paper industry	Shleev *et al.,* 2006
5.	*Cariolus hirsutus*	Biosensor for the determination of phenolic compounds in effluent samples from paper mills	Freire *et al.,* 2002
6.	*Coriolus hirsutus*	Biosensor for the determination of phenolic compounds in real water samples	Marko-Varga *et al.,* 1995
7.	*Trametes versicolor*	Biosensor for phenol monitoring in wastewater	Freire *et al.,* 2001

Laccase not only helps in the degradation of toxic phenols and pollution control but also has various applications in different fields i.e., agriculture, pesticides and break down of agrochemicals, lessening their damaging effects on the environment and biofuel production they help in breaking down the lignocellulosic compounds for producing biofuel. Laccase from *Trametes versicolor* potentially decomposes pesticides, insecticides, fungicides and herbicides (Virk *et al.,* 2012).

LACCASE IN THE PAPER INDUSTRY

Bio-pulping paper from wood requires separating the wood fibers turning them into paper. Lignin in wood binds the fibers together. These fibers can be separated by breaking down and removing lignin (chemical pulping) or by physical separation of the fibers (mechanical pulping). Chemical pulp and mechanical pulp have different industries. Many paper products contain two pulps in proportions based on the needs of the product. Mechanical pulping is cheaper than pulping due to its high yield (up to 95% by weight of starting material, compared to usually less than 50% in chemical pulping of wood) and capital cost. However, the high lignin content of the fiber material reduces the paper quality; Because the fibers are not easy to combine, the paper is less durable and the skin turns yellow in the sun. Furthermore, mechanical pulping is more expensive because it needs a lot of electrical equipment. Therefore, in the paper industry, laccase plays an important role by binding fibres together and is comparatively more affordable than mechanical pulping (Alcalde, 2007; Chauhan *et al.,* 2017; and Kuhad *et al.,* 1997).

BIO-BLEACHING BY LACCASE

Wood fiber has many layers composed mainly of cellulose, hemicellulose, and lignin. Lignin is an insoluble complex polymer of phenolic compounds. During the pulping process, up to 90% of the lignin is dissolved and removed. Residual lignin is the main cause of residual colour in pulp and has to be eliminated by bleaching or oxidative destruction. The whitening process requires the use of harsh chemicals and energy. Traditional pulp de-lignification or decolorization methods involve chlorine or oxygen-based chemical oxidants (such as ClO_2 and O_2). (Bajpai and Bajpai, 1992). Although effective, this process has disadvantages such as wastage of chlorinated by products or loss of cellulose fiber strength. Microbial or enzymatic de-lignification systems overcome these disadvantages and can be easily adapted to existing production lines, making them attractive. Filamentous fungi can degrade lignin under the action of various secreted enzymes. Among them, the application of laccase in pulp bio bleaching has become very popular. It is thought that during the degradation of lignin, laccase breaks down small phenolic lignin fragments, which then react with the lignin polymers, causing them to break down. Alternatively, a combination of materials can be provided to accelerate the de-lignification process. (Biermann, 2018).

LACCASE IN WASTE DETOXIFICATION AND DECONTAMINATION

Laccase has been used for several aromatic xenobiotics or for oxidative detoxification and bacteria found in industrial wastes and contaminated soil or water. Laccase catalysis can lead to direct degradation or polymerization/immobilization. Direct degradation include direct dechlorination, aromatic ring cleavage, mineralization of PAHs (Polycyclic Aromatic Hydrocarbons), decolorization of pulp or cotton mill waste, and bleaching of textile dyes. The process involves polymerization of the

organisms themselves or copolymerization with other non-toxic materials, such as humic materials. Collected contaminants often become insoluble or immobilized so they are easily removed by adsorption, sedimentation, or filtration. (Xu, 1999)

LACCASE IN DECOLORIZATION OF DYES

Approximately 10,000 different dyes and pigments are produced worldwide each year and are widely used in the printing and dyeing industry. Total paint production in the world is approximately 800,000 tons per year, and at least 10% of waste paint ends up in the environment through landfills. (Palmieri *et al.,* 2005). The majority of the dyes are very stable to light, heat, and bacterial contamination, making them resistant. These industrial waste products exhibit elevated biological and chemical oxygen demands (BOD and COD), suspended particles, and vivid color. They are hazardous. These different dyes and colours can be removed with the help of the laccase enzyme which catalyzes the oxidation of molecules of dyes (Gupta *et al.,* 2024).

LACCASE IN THE TEXTILE INDUSTRY

Laccase is reported to prevent back staining in dyed or printed textiles. As part of the cleaning solution, laccase quickly bleaches the exposed dye, thus reducing the processing time, energy, and water required to complete the document. Laccase-catalysed bleaching of textile dyes can also be used in the finishing of dyed cotton fabric. Another interesting application of laccase in this field is oxidative transformation and subsequent binding of dye precursors to the collagen matrix. Soluble dye precursors can be adsorbed, oxidized, and polymerized to form the tanning effect during catalysis by laccase. This process can increase dyeing efficiency, reduce costs (by using cheaper precursors), or provide better skin quality (Xu, 1999; Yesilade *et al.,* 2002).

MEDICAL AND PERSONAL CARE APPLICATIONS OF LACCASE

Poison ivy dermatitis (caused by skin contact with poison ivy, poison oak, or poison sumac) is usually caused by urushiol, a catechol-derived toxin. However, oxidized urushiol, an o-quinone derivative, is nontoxic. It has been demonstrated that laccase detoxifies, oxidizes, and polymerizes urushiol, lessening the impact of toxic ivy dermatitis (Judy, 2018). Another application of laccase in this field is laccase-based *in situ* iodine production. Laccase oxidizes iodide to produce iodine, a reagent commonly used as an antibiotic. Application of the laccase-iodide salt binary iodine production system (for sterilization) may be more effective than direct application of iodine. First, iodized salt is more stable and is safer to store, handle, and transport. Second, the release of iodine in the laccase-iodide system can be easily controlled (by adjusting the laccase concentration, etc.). The system can be used in a variety of commercial, medical, home, and personal care applications, such as disinfection of drinking water and swimming pools and small-scale disinfection (Judy,2018).

LACCASE IN THE FOOD INDUSTRY

The food industry has made extensive use of laccase, an adaptable enzyme, mainly because of its capacity to oxidize phenolic chemicals. Due to its properties, it has been extensively used in the food industry. Enzyme increases food stability, extending shelf life and preserving quality after lowering phenolic concentration. Moreover, laccase is employed in the manufacturing of hypoallergenic products by decreasing the concentration of allergic components, enhancing flavor by altering particular flavor precursors, and producing functional foods to boost antioxidant qualities (Couto and Herrera 2006. Some of the important applications in the food industry are mentioned below-

1. Wine making

Wine Stabilization: Wine stabilization is one of the main uses of laccase in the food industry. Fruit juice and fruit juice are mixtures of different substances such as ethanol, organic acids, salts, and phenolic compounds. Alcohol and organic acids determine the aroma of wine, and the phenolic compounds in it determine its color and taste. The organoleptic properties of new wine should not change before drinking; Due to complex events in which polyphenols play an important role, enzymes containing iron, copper, and aldehydes, amino acids, and proteins catalyse the oxidation occurring in fruit juice and wine, causing turbidity, darkening of color and changes in aroma and taste. This oxidation phenomenon is called Madeirization; (**https://www.sciencedirect.com/topics/food-science/madeira-wine**) and the laccase enzyme is widely used for this purpose.(Mayolo-Deloisa *et al.,* 2015). Laccase are used for the removal of O^2_2 from beer at the end of production process, thus

enhancing the storage life of beer (Claus *et al.,* 2007). Laccases are also helpful in stabilizing the fruit juices (Lacasse and Baumann 2012).

2. **Tea industry**

Laccase is an essential component in the tea business that improves the stability and overall quality of tea products. Laccase decreases unwanted bitterness and astringency by oxidizing phenolic chemicals, resulting in a smoother and more acceptable flavor by altering phenolics, it helps to prevent browning in green and white teas while maintaining the tea's look and colour. By dissolving substances that cause haze, laccase aids in the clarity of tea extracts and produces clearer drinks. It also strengthens tea's antioxidant qualities, which increases the health advantages of the beverage. Laccase increases the tea's shelf life by stabilizing the tea and halting spoiling. It also ensures a cleaner taste by reducing off-flavors that may arise during processing. (Xu *et al.,* 2020).

3. Baking

In bread making, it is a practice to add bread and/or dough to improve the product added to the bread, which makes the bread better, increasing its volume, flavor, and freshness, as well as improving the dough (Bhamare and Sayyed, 2016). Enzymes (amylase, protease, cellulase, etc.) are used to improve dough and/or bread. (Si, 1994) stated that when laccase is added to the dough used in the production of bakery products, it can create an oxidative effect on the dough, thus gluten free in cookies and/or bakery products. The use of laccase improves the performance of the dough by increasing the volume of the baked product, improving its structure and softness, and making it stronger, firmer, and less sticky. It has been found to work especially well for dough when low-quality flour is used (Osma *et al.,* 2010).

CONCLUSION

In conclusion, the versatility and efficiency of laccase enzymes derived from mushrooms have significant potential across various industrial and environmental applications. These enzymes, capable of degrading a wide range of phenolic and non-phenolic compounds, are particularly valuable in bioremediation efforts, including the treatment of industrial wastewater from the textile, pulp, and paper industries. Their environmentally friendly nature facilitates effective waste management solutions and contributes to mitigating environmental pollution. Beyond bioremediation, laccase enzymes find important applications in food processing, enhancing product quality and sustainability in processes such as wine stabilization and bread making. Mushrooms, with their metabolites like laccase enzymes, play a crucial role not only in industry but also in ecological and nutritional contexts. They are valued for their nutritional, pharmaceutical, and economic benefits, providing essential vitamins and minerals while being low in calories. Their unique properties and ability to form symbiotic relationships further enhance their ecological significance. The increasing consumption of mushrooms highlights their growing importance in sustainable practices and health benefits. The ongoing research and development in laccase applications promise further advancements, positioning these enzymes as critical tools in promoting environmental sustainability and industrial innovation. Overall, the laccase enzyme from mushrooms emerges as a diversified biotechnological tool, fostering sustainability and efficiency across diverse sectors, from industrial waste management to food processing and ecological preservation.

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