# **An Overview on Laccases: Production & Industrial Applications**

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## **Abstract**

Laccases have drawn a lot of attention from researchers in recent years. This makes them an excellent choice for use in a variety of biotechnological processes. These enzymes possess capacity to oxidize substances associated to lignin includingextremely durable environmental contaminants. The purification the commercial byproducts,primarily from the textile, petrochemical, and paper and pulp sectors; use as a medical instrument for diagnosis along with bioremediation harmful chemicals and explosives are some examples of these applications. The present review explores the different strategies of laccase production for obtaining maximum yield along with their uses and future expansion in several industrial sectors.

**Keywords:** Laccases, biodegradation, delignification, fermentation, biofuel, pulp and paper effluents.

## **Introduction**

Although oxidation processes are necessary in many different industries, most traditional oxidation methods have the following shortcomings: they use chemicals that are dangerous to the environment and have non-specific or unwanted side reaction. This has sparked research into novel biologically-based oxidation technologies, like enzymatic oxidation (Rochefort et al., 2008). The aforementioned systems outperform chemical oxidation in the following ways: enzymes operate as specialised, Catalysts that degrade naturally and enzyme reactions take place in safe circumstances. The industries of paper and pulp, clothing, and food are just a few of the industrial sectors where enzyme oxidation approaches have potential (Williamson et al., 1994). The most fascinating enzymes recycle utilizing oxygen in the gas phase as an electron acceptor. In light of the above statement stated, laccase is a very intriguing biocatalyst. The laccase protein is a smultimeric glycoprotein that typically has four copper atoms distributed among each monomer's three redox sites. This enzyme catalyses the reduction of molecular dioxygen to water along with the oxidation of certain compounds among these are lignins, amino-phenols, polyphenols, polyamines, and aryl diamines (Claus et al., 2002).Since laccases have lower reported redox potentials than non-phenolic chemicals, they cannot oxidise those molecules. Subsequently, was demonstrated that laccases were also capable of oxidising non-phenolic structures in the presence of smaller molecules that might serve as mediators for transport of electrons. Subsequently, was shown that laccases may oxidise non-phenolic compounds when small molecules are present that could serve as mediators for transport of electrons, broadening the variety of substances that these enzymes can oxidise (Couto et al., 2006). Numerous procedures, including pulp delignification, have utilised laccase-mediated systems (LMS). For the aforementioned uses, a number of organic and inorganic substances have been reported to be efficient mediators. These include ferrocyanide, N-hydroxy compounds, and derivatives of the phenol and thiol aromatics, respectively ((Rochefort et al., 2008). The LMS increases dye decolorization and decolorized several dyes that are immune to laccase degradation.TheLMS's applications, also include methods for pulp bleaching, fabric biofinishing, and safeguarding the environment. Regarding the substantial research on LMS, there are still issues with toxicity, cost, and mediator recycling that need to be resolved (Couto et al., 2006). In recent years, laccases have been evaluated multiple times, usually focusing on specific details. Excellent overviews of the laccases' enzymology and electron transport mechanism have been published (Selinheimo et al., 2006). Highlighting potential commercial and biotechnological uses for laccase enzyme is the goal of this review.

## **Sources of laccases**

Numerous microorganisms, including bacteria and fungi and even certain plants are involved in production of laccases under different physical, physiological and environmental conditions. Maximum studies are reported about production of laccases by variety of fungi, such as, white rot fungi, brown rot fungi, basidiomycetes and ascomycetes groups which are involved in degradation and decomposition of complex organic compounds(see table no. 1). Although bacteria normally produce lesser amounts of enzymes than fungi, some are known to produce laccase as well. As reported in table No. 1, plenty of bacteria, such as, *Pseudomonas, Enterobacters, Alcaligens, Bacillus* etc., including a novel species, *Bhargavaea beijingensis*

are responsible for production of laccases with immense industrial applications.



#### **Table.1. Recently reported sources and applications of Laccases**



## **Laccase production & optimization**

When laccase was discovered for the first time in respective domains, its functions must have been native in those respective organisms. Such functions included lignification and wound healing in plants (Bao et al., 1993), iron metabolism and maintaining homeostasis in fungus (Thurston, 1994), cuticlesclerotization in insects (Anderson, 1985; 1990) and pigmentation and morphogenesis in bacteria (Singh et al., 2011). Scientists would have researched and experimented with the same.

 But now, as we see that laccase has countless industrial roles, researchers are trying a lot of optimization techniques to manufacture and dig out laccase from diverse organisms. Laccases have an expansive spectrum of applications in distinctive fields. Therefore, there is a constant need for production of it in immense amounts, that too with a cost-effective and eco-friendly approach (Mate & Alcalde., 2017; Arregui et al., 2019). The improvement of certain properties is also enviable to achieve commercially attractive and enhanced catalytic performance.

 Laccase production can be achieved either through (a) submerged fermentation, and (b) solid-state fermentation.

## **(a) Laccase production through Submerged fermentation**

 Submerged fermentation (SmF) is the process in which decomposition (anaerobic and/or partially anaerobic) of substrates is accomplished by an enzyme produced by microorganisms in the presence of abundance of free water (Mussatto and Teixeira, 2010). This technique iswidely used in laccase production; like Bakkiyaraj (et al., 2013) achieved 9300 U/L laccase production using wheat bran as the carbon source. Researchers have reported that the optimization of medium components is one of the promising methods to improve enzyme production in submergedfermentation. To give an instance Rodrigues (et al., 2019) have tested the production of laccase by the basidiomycete fungus *Ganoderma lucidum* in the presence of different inducers and concluded that the presence of 2 mM ferulic acid resulted in the highest value of laccase activity on the seventh day of cultivation (Xu et al., 2020). This method is usually employed when laccase is intended to be extracted from a bacterial source. Some potential bacteria have shown maximum laccase production in submerged conditions due to their growth suitability and proper nutrient availability (Kumar et al., 2021). Forcase, VikasSharma (et al., 2021) extracted a thermotolerant laccase enzyme from *Bacillus licheniformis* VNQ using submerged fermentation. It had a molecular mass of ∼48 kDa. It required optimum temperature and pH as 55°C and 5.0, respectively and possessed a half-life of 4 hours at 70°C. But it is also employed for extraction from fungal species like using SmF, the highest laccase activities (386 U/L and 1216 U/L) were obtained from *T. trogii*grown in a medium containing pulverized apricot seed shell by Birhanli and Yeşilada (2013). Ghosh&Ghosh (2017) achieved enhanced laccase production of about 4.6-fold higher than the unoptimized media.

The laccase production under submerged fermentation still cannot satisfy the demand due to its low concentration(Xu et al., 2020). Therefore, new techniques like Solid-state fermentation (SSF) have been evolved.

## **(b) Laccase production through Solid-State Fermentation**

Solid-state fermentation (SSF) refers tothe process of the microbial fermentation that involves insoluble solid materials as support and occurs in the absence or near the absence of free water (Nandal et al., 2013). This method may also be seen in some natural biological processes like composting (Rodriguez-Couto, 2018; Pourkhanali et al., 2021). Singh&Abraham (2013) studied laccase production by *Fusarium sp.* and *Aspergillus sp.* grown in compost culture.

This method has some advantages to offer over SmFlike high volumetric productivity (Duenas et al., 1995), effective utilization of agro-industrial wastes as substrates (Murugesan et al., 2007), no requirement of complex mechanical and control systems and provides high laccase yield with low energy inputs (Chenthamarakshan et al., 2017; Sadeghian-Abadi et al., 2019). Moreover, SSF provides culture conditions similar to natural habitats, which is beneficial for organism growth and laccase production. Sharma (et al., 2019) reported production of laccase from *Ganoderma lucidum*under SSF to be more stable than the in vitro enzyme.

Moreover, low water availability decreases the risk of bacterial and yeast contamination and the absence of free moisture increases aeration, favoring oxidative metabolism. For instance, Pourkhanali (et al., 2021) produced and optimized laccase of 55 kDa from *Galactomyces geotrichum*usingthis method. They provided optimal cultural conditions as moisture content (80%), fermentation time (14 days),  $CuSO<sub>4</sub>·5H<sub>2</sub>O$  as the inducer (300 μM) and glucose as a co-substrate (5 g/L). The maximum laccase activity obtained was 52.86 (U/g of the dry substrate). Debnath (et al., 2021) focused on enrichment of the laccase production from Phoma herbarum isolateKU4 using Solid-State Fermentation providing cheap agro-industrial wastes as substrates. They obtained 79008 U/g laccase production, which is approximately six-fold enhanced production compared to the unoptimized condition. Even bacterial cultures such as of *Bacillus amyloliquefaciens* can be used for spore laccase enzyme production using SSF, as reported by El-Bendary (et al., 2021).

## **Effect of Inducers**

Researchers have reported that the productions of laccaseare affected by multiple fermentation factors such as medium composition, carbon and nitrogen sources, pH, incubation temperature, etc. (Patel et al., 2009; Xin and Geng 2011; Akpinar & Urek, 2017).Additionally, lots of compounds have been widely used to fire uplaccase productions by means of the use of inducers (Patel et al., 2009). For instance, Rodrigues (et al., 2019) found sawdust resulted in the highestlaccase activity in *P. taeda*on the fourth day of cultivation. However, on the seventh andeighth day, the extracts from the cultivation in thepresence of ferulic acid showed the highest values ofenzymatic activity, 49 and 44U/L, respectively. Copper hasbeen reported to be a strong laccase inducer by manyresearchers (see table 2). In *Ganodermasp.* rckk-02, laccase yield was optimized with wheat bran substrate and the presence of  $Cu^{2+}$  ions under SSF (Sharma et al.,2015).Used tea leaves were also evaluated as the inducersof versatile peroxidase production from *Pleurotus eryngii* by SSF(Lu et al., 2019).Vinasse is an agro-industrialwaste from sugarcane industry that can be used as inducer for laccase production (see table no. 2). It has been used for laccase production from *T. versicolor*through liquid fermentation (Pinheiro et al., 2020).



## **Table 2: Production & optimization of laccases under physical and chemical parameters for maximum yield**

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## **Possible applications of laccase biocatalyst in industry and biotechnology**

Laccases are employed in the following industries:

## **Food Industry**

Laccases can be used in specific procedures that improve or change the visual appeal of food or beverage colour. This makes the eradication of unfavourable phenolics, which cause Clear juices start to turn brown, cloud up, and becomes turbidfrom fruits and beverages, which is a fascinating use of laccases (Khatami et al., 2022) Biopolymers can be cross-linked by these substances. So, laccases are currently being studied in baking. Recent reports demonstrated that a White-rot fungus laccase from Trametes hirsuta boosted dough's maximal resistance while lowering its extensibility in dough made with gluten and wheat. The possible uses of laccase in the food sector have recently been discussed (Strong & Claus, 2011). These include biosensor applications, alcohol production, ascorbic acid measurement, sugar beet pectin gelation, biological remediation, and food preparation. To increase the commercial use of this enzyme, they proposed that more research be done on laccase production and low-cost immobilisation procedures (Couto et al., 2006).

## **Paper and pulp industry**

Separation and lignin degradation in wood pulp are necessary steps in the industrial manufacture of paper. Environmental concerns call for the replacement of traditional, harmful, chlorine-based delignification techniques. Although industrial-scale oxygen delignification processes have been created, pre-treating pulp from wood with ligninolytic agents may give gentler, clearer delignification processes that also preserve the cellulose's inherent properties (Camarero et al., 2004). Despite extensive research being conducted to create alternative bio-bleaching systems, few enzymatic therapies exhibit the brightening capabilities of modern chemical bleaching procedures. A rare exception to this norm is the development of LMS delignification technology for kraft pulps. Additionally, compared to laccase, lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) are less accessible and more challenging to control (Couto et al., 2006). LMS has already found use in industry, such as the Lignozym®-process. Several studies employed the LMS to biobleach pulp. However, much of the biobleaching research has focused on wooden pulps, and less is known about how well the LMS works with non-wood pulps like those used to manufacture specialty papers (Fernandez et al., 2013). LMS also has the capacity to remove substances that give highquality flax pulp its color. They showed that it is feasible to produce these premium paper pulps without utilizing chlorine-containing chemicals by substituting LMS (Camarero et al., 2004). The ability of laccases to produce radicals that react in lignin may be used to precisely change wood fibers. Laccases, for

instance, may be used in the catalytic attachment of fibers during the creation of composite lignocellulose materials, such as fiberboards. It has been suggested that laccases can be used to activate the fiber-bound lignin during the creation of composites, resulting in the production of boards with outstanding mechanical properties without the usage of risky synthetic adhesives (Camarero et al., 2004). Another method for improving the chemical or mechanical properties of goods made from fibers is to functionalize lignocellulosic fibers using laccases. According to early research, laccases can graft several phenolic acid derivatives onto the fibers of kraft pulp (Fernandez et al., 2013).

## **Textile Industry**

The textile sector occupies two thirds of the dyestuffs market and uses plenty of chemical substances and water to wet process cloth. These enzymes are employed in the pretreatment of textiles to remove lignin and other impurities from natural fibers like cotton and linen. This bio-bleaching process enhances the whiteness and quality of textiles, reducing the need for harsh chemicals like chlorine bleach (Strong & Claus, 2011). They are also involved in activating and fixing natural dyes onto textiles, making the dyeing process more sustainable and eco-friendlier. This approach eliminates the need for chemical mordants, which can be environmentally harmful. These biocatalysts are also employed in removing impurities, such as grease and lanolin, from wool fibres during the scouring process (Zille, 2005). This results in cleaner, softer, and more eco-friendly wool products. Laccases can be used in fabric softening treatments, replacing or reducing the use of chemical fabric softeners. This can lead to textiles with a softer and smoother (Selinheimo et al., 2006;Rochefort et al., 2008).One of the primary uses of this enzyme in the textile industry is for the treatment of textile effluents. They are capable of breaking down and decolorizing synthetic dyes that are often used in textile dyeing processes. This helps in reducing the environmental impact of textile wastewater by removing colour and improving water quality (Rochefort et al., 2008).

## **Waste valorisation and biofuel production**

Laccases are employed in degradation of lignin and other organic components in agricultural residues, such as crop stalks and corn cobs. This degradation process is responsible for converting agricultural wastes into valuable resources such as biofuel production (Asgher,et al., 2014). Moreover, biorefineries generate various waste streams rich in lignocellulosic materials. Laccases can be used to extract valuable compounds from these waste streams or further break down lignin for utilization in the production of biobased products (Curran et al., 2022). These biocatalysts are also involved in the pretreatment of lignocellulosic biomass to modify the lignin structure, making it more accessible to enzymatic hydrolysis which leads to enhancement in the efficiency of subsequent steps in the biofuel production process. Laccases are also employed in improvedproduction of ethanol from lignocellulosic biomass, such as agricultural residues and forestry waste (Gao et al., 2018). By breaking down lignin and other inhibitory compounds, laccases enhance the fermentability of biomass and increase ethanol yields (Gonçalves et al., 1025).They are also used to pretreat lignocellulosic feedstocks for the production of biodiesel. By breaking down lignin, they improve the accessibility of cellulose and hemicellulose, which can then be converted into biodiesel precursors (Senthivelan et al., 2016).These enzymes are also employed in the production of advanced biofuels, such as lignin-derived biofuels and cellulosic ethanol, which offer higher energy yields and reduced greenhouse gas emissions compared to traditional fossil fuels (Gonçalves et al., 2015).

## **Conclusions**

In conclusion, microbial laccases are versatile enzymes with a wide range of applications in various industries and environmental processes. These enzymes are produced by microorganisms, including fungi,

bacteria, and some algae, and possess several key characteristics and features that make them valuable. Microbial laccases exhibit a broad substrate range, allowing them to oxidize various compounds, including phenolic and non-phenolic substances. This versatility is a key asset in their industrial and environmental application. These biocatalysts support sustainability goals by reducing the environmental impact of various industrial processes, minimizing waste generation, and enabling the use of renewable resources.

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