



# A Review: Optimization of Fungal Laccase Production and their Potential Application in Effluents Treatment

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**Abstract:** Due to globalization, urbanization, and industrialization water pollutants and contaminants rise in water bodies which distort an ecosystem and impacting human and animal health. To eliminate various contaminants from water bodies, so many techniques and strategies have been designed. Nowadays laccase enzymes pay attention due to their high potentiality, substrate specificity and atmospherically constitute benign reaction properties and are used to remove environmental and industrial pollutants. Fungal laccases are enzymes with a lot of promise for removing harmful phenolic and non-phenolic chemicals from industrial effluents, as well as for degrading resistant xenobiotics. There have recently been a slew of studies on the advancements in the creation of laccase enzyme by modification of growth and optimization conditions and the discovery of new endophytic strains for the production. The following evaluation of the literature relates to optimising endophytic laccase production and also provides new insights for biotechnological applications in environmental protection, such as wastewater/effluents treatment.

**Index Terms:** Urbanization, Industrialization, Endophytic Laccase, Phenolic compounds.

## I. INTRODUCTION

Phenolic compounds are widespread in nature. Phenol is present in vegetables and fruits. After breakdown, natural organic molecules such as humic substances, lignins, and tannins produce phenols. Phenols are persistent pollutants that enter waterways through the effluents of industrial activities such as petrochemicals, pulp, paper, paints, textiles, coal refineries, medicines, and resin manufacturing, including the formation of phenol (Bollag, *et al.*, 1988; Viswanath, *et al.*, 2008; Shraddha, *et al.*, 2011). Industrial discharges produced by various industries in huge quantities and their disposal create a big problem in the environment. The untreated discharge of phenol

and phenolic chemicals may pose substantial health concerns to humans, animals, and aquatic ecosystems. For an imperishable environment, International regulatory authorities such as the EPA have set strict discharge limitations for phenols (Viswanath, *et al.*, 2008). For example, phenol is less than 1 ppb has set an incredible clarity of surface water by the EPA (Shraddha, *et al.*, 2011). The Contagiousness levels usually are in the 9–25 mg/L range for the living form of life (Bollag, *et al.*, 1988). Both acute and chronic kinds of perilous health effects can possess phenol and phenolic compounds. Human exposure to phenol at LD value for Long-term use can cause dyspnea, muscular dizziness, tremor, coma, respiratory malfunction (apnea), skin, eye, and mucous membrane irritation, and more. Chronic consequences of phenol exposure in animals include anorexia, weight loss, diarrhoea, vertigo, salivation, and dark urine colouring, as well as irritation of the gastrointestinal, cardiovascular, liver, renal, and central neurological systems. (Thurston, 1994; Viswanath, *et al.*, 2008; Shi, *et al.*, 2013). Animal studies have revealed that the progeny develop abnormally. To protect the environment from the harmful effects of phenol, wastewater effluents containing phenolic compounds must be treated before release.

To detoxify these industrial wastes effluents and prevention of their harmful effects on the environment, laccase can work as a good remedy to solve it. Laccases have lately gained popularity because to their prospective applications in the detoxification of recalcitrant contaminants and bioremediation of phenolic and non-phenolic substances (Singh, *et al.*, 2009; Desai, & Nityanand, 2011; Singh, *et al.*, 2011). Their ability to act on the diversity of substrates as well as modify a variety of contaminants into nontoxic compounds (reactions with great efficiency, selectivity, and environmental friendliness). Because

their cosubstrate oxygen is usually present in their environment, laccases do not require the inclusion of a low molecular weight cofactor or its production. Because most laccases are extracellular, they are easy to purify; they also have a high level of stability in the extracellular environment; and laccase expression is inducible in most fungal species, contributing to their stability. The development of laccase-based biocatalysts that are highly efficient, long-term, energy-efficient, and biodegradable, as well as industries that is ecologically benign. Laccases promise in biotechnological and environmental applications has sparked a desire to find promising laccases in large quantities, and the need for this enzyme necessitates a cost-effective production procedure (Riva, 2006).

The study's goals were to find out more about optimization of laccase production from endophytes and their use to the total phenol content and chemical oxygen demand in diverse wastewaters release from industries (thus lowering toxicity), raising the pH to a level suitable for further treatment, and lowering the colour. Parallelly laccase production at a greater concentration, secondary objective was to bioremediate wastewater/ industrial effluents.

## II.SOURCES OF LACCASES

The study of laccase (EC 1.10.3.2, p-diphenol, dioxygen oxidoreductase) has been done since the nineteenth century. Laccase enzyme has the potential ability of oxidation. Laccase comes under a small group known the blue multicopper. It belongs to the group of enzymes that create reactive radicals with inherent characteristics (Bertrand, 1985; Ranocha and coworkers, 1999; Battistuzzi, *et al.*, 2003). Laccase was discovered in the exudates of the Japanese lacquer tree, *Rhus vernicifera*, by Yoshida in 1883. Afterward, laccases have been discovered from numerous plants (Johnson, *et al.*, 2003) but plenty of oxidative enzymes presence in crude plant extracts creates difficulties in isolation and purification, which is the reason behind the lack of information about plant laccases. Plant laccases are found in the xylem, where they catalyse a stage of the lignification reaction that occurs earlier (Gavnholt & Larsen, 2002; Hoopes & Dean, 2004). Plant laccase is less common than fungus laccase (Mayer & Staples, 2002; Moin & Omar, 2013).

Bertrand and Laborde in 1896 searched firstly laccase was a fungal enzyme. Laccases are glycoproteins present in a wide range of organisms, including plants, fungus, bacteria, and insects. A typical fungal laccase is a 60-70 kDa protein with an acidic isoelectric point of roughly 4.0 (Johnson *et al.*, 2003). Laccase activity has been found in several ascomycetes and basidiomycetes fungus species, and the enzyme has been isolated from many of them, including *Melanocarpus albomyces* (Kiiskinen, & coworkers; 2002), *Cerrena unicolor* (Kim *et al.*, 2002), *Magnaporthe grisea* (Iyer & Chattoo, 2003), *Trametes versicolor* (Minussi & coworkers; 2007), *Trichoderma reesei* (Levasseur, & coworkers; 2010) and *Xylaria polymorpha* (Nghi

& coworkers; 2012). Laccase synthesis has also been documented for several soil ascomycete species belonging to the genera *Aspergillus*, *Curvularia*, and *Penicillium* (Scherer & Fischer, 1998) as well as some freshwater ascomycetes (Junghanns, *et al.*, 2005). Yeasts are able to produce true laccase which can oxidise phenols and derivative of phenols, but can't oxidise tyrosine (De Jesus, *et al.*, 2009) and provided resistance from fungicides (Ikeda, *et al.*, 2001). Laccase has already been found in varying amounts in all white-rot fungi species (Hatakka, 2001), and most of them are efficient lignin degraders (Kiiskinen, *et al.*, 2004). Laccase producing basidiomycetes were *Agaricus bisporus* (Wood, 1980), *Botrytis cinerea* (Marbach *et al.*, 1984), *Phlebia radiata* (Niku-Paavola, *et al.*, 1988), *Pleurotus ostreatus* (Sannia *et al.*, 1986), *Trametes versicolor* (Rogalski, *et al.*, 1991) and *Coprinus cinereus* (Schneider, *et al.*, 1999). Laccases have not only been found in plants, bacteria, and fungi, as well as in insects, where they are involved in cuticle sclerotization (Dittmer *et al.*, 2004). Laccases are now used by termites to make lignin modifying enzymes (lignases). The fungi isolated from the nests of three taxa of fungus-eating termites (fungus combs), *Macrotermes*, *Odontotermes*, and *Microtermes* (fungus combs), had high laccase activity against DMP (2, 6-Dimethoxyphenol) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Taprab & coworkers; 2005; Scharf *et al.*, 2011; Ni & Tokuda, 2013). 127 endophytic fungal strains have been isolated from eucalyptus trees and, out of them, 21 fungal strains have been screened for ligninolytic enzyme production (Brijwani, *et al.*, 2010).

Laccases have a role in lignification in plants, and they've been linked to a variety of cellular activities in fungi, including delignification, sporulation, pigment generation, fruiting body development, and plant disease. Laccases are capable of enhancing the quality of pulp either by functionalizing lignocellulosic fibers or by forming reactive radicals with lignin. Laccases can also use their polymerization and depolymerization activities to remove colourful and hazardous substances generated as effluents from various industries and render them benign (Mayer, & Staples, 2002).

Laccases have been found in only a few bacterial species. "*Azospirillum lipoferum*" was the first bacterial laccase isolated from plant root (Givaudan, *et al.*, 1993) which is involved in the synthesis of melanin (Faure, & coworkers, 1994). Laccases have also been found from *Marinomonas mediterranea* which contain six putative copper-binding sites (Sanchez-Amat, *et al.*, 2001). Laccase from *Bacillus subtilis* CotA is a thermostable laccase that plays a role in pigment formation in the endospore coat (Martins & coworkers, 2002). *Streptomyces cyaneus* (Arias *et al.*, 2003) and *Streptomyces lavendulae* (Suzuki *et al.*, 2003), and some *Bacillus* sp. laccases produced from endospores were involved in phenol degradation (Naclerio, *et al.*, 2010; Singh, *et al.*, 2011) whereas Laccases from *Bacillus licheniformis* are

involved in phenolic acid dimerization (Koschorreck, *et al.*, 2008). In this way, bacterial laccases are intracellular or periplasmic proteins that showed different kinds of activity in different bacterial sources.

### III. MECHANISM ACTION OF LACCASE

A wide spectrum of aromatic chemicals, including polyphenolic compounds, are catalysed by the laccase enzyme (Bourbonnais, & Paice, 1990), methoxy-substituted monophenols, and aromatic hydrocarbon amines, by reducing one molecule of oxygen to water, followed by one-electron oxidation (Bourbonnais, *et al.*, 1995). An oxygen-centered free radical is produced during the oxidation process, which can subsequently be converted to quinone utilising an enzyme-catalyzed reaction. Using UV/visible and electron paramagnetic resonance (EPR) spectroscopy, monomeric laccase molecules containing four copper atoms can be divided into three groups (Palmieri, *et al.*, 1998). Type I copper (T1) provides the enzymes a bright blue colour at 600 nm and is EPR-detectable, whereas type II copper (T2) appears uncolored but is EPR-detectable, and type III copper (T3) is made up of two copper atoms with a weak UV absorbance but no EPR signal (Bourbonnais, & Paice, 1996). The copper locations T2 and T3 are close together, forming a trinuclear core (Palmieri, *et al.*, 1998) that is crucial in the enzyme's catalytic mechanism (Bourbonnais, & Paice, 1996).

The reaction is catalysed by laccase in three steps: (1) type I Cu reduction by the substrate (2) type I Cu to type II Cu and type III Cu trinuclear clusters electron transfer (3) At the trinuclear core, oxygen is reduced to water (Gianfreda, & coworkers 1999) and catalyses a wide variety of aromatic compounds (Couto & Toca-Herrera, 2007). The mediators such Low molecular weight chemical compounds such as 1-hydroxybenzotriazole (HOBt), N-hydroxyphthalimide (NHPI), and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) can be used to broaden the laccase substrate range to include non-phenolic substances. Laccase can be oxidized mediators and firstly form very reactive radicals of cation oxidising non-phenolic chemicals that laccase alone cannot oxidise (Gochev & Krastanov, 2007). Only laccase binds to the phenolic components of lignin, causing C $\alpha$  oxidation, C $\alpha$ -C $\beta$ , and aryl-alkyl bond breakage.

### IV. SCREENING OF LACCASE PRODUCING ORGANISMS

It is critical to screen laccase-producing fungal species and their variants in order to choose laccase-producing fungal strains that will work in industrial applications (Monteiro, & De Carvalho, 1998). Novel laccases with varied substrate specificities and enhanced stabilities must be discovered for industrial uses. Qualitative screening of laccase production has been done by plate method on solid selective media that carries coloured indicator chemicals can be detected by visualization

(Nishida, *et al.*, 1988) whereas liquid culturing has also been subjected to quantitative investigation via enzyme activity assays (Luterek, and coworkers, 1997). Gallic and tannic acid (Harkin & Obst, 1973) are screening reagents used in old days. Nishida *et al.* (1988) used synthetic phenolic reagents like guaiacol and syringaldazine, whereas Raghukumar, *et al.* (1999) applied the polymeric dyes Remazol Brilliant Blue R (RBRR) and Poly R-478 for screening purposes. A favourable reaction using guaiacol is shown by the production of a red - brown zone (Nishida, *et al.*, 1988), whereas a significant result using tannic and gallic acid is indicated by the formation of a dark-brown coloured zone (Harkin & Obst, 1973).

### V. LACCASE PRODUCTION OPTIMIZATION PARAMETERS

Laccase is enzyme produced during secondary metabolism and released into the medium extracellularly by filamentous fungi (Dong, *et al.*, 2005). Enzyme expression is correlated with Culture conditions and medium composition. Several fermentation parameters like, sources of carbon and nitrogen, pH, temperature, medium, incubation duration, enhancer concentration (Palmieri, & coworkers, 2000), aeration (Dekker & Barbosa, 2001), and other factors, such as the kind of cultivation (submerged or solid-state), can influence laccase production (Monteiro, & De Carvalho, 1998).

#### A. Effect of Carbon & Nitrogen Source on Laccase Production

Carbon is the primary source that liberates energy for the growth and development of an organism. The most readily usable carbon source is glucose reported for laccase production in various fungal cultures. Various concentration of glucose has been described for maximum laccase production such as *Coriolus versicolor* at (10 g/L) (Collins, & Dobson, 1995), *Trametes versicolor* (20 g/L) (Battistuzzi, *et al.*, 2003). *Ganoderma lucidum*, (10 g/L) (Perumal, 1997), *Lentinula edodes* and *Grifola frondosa* (10 g/L) (Arora, & Rampal, 2002; Cavallazzi, & coworkers, 2005), *Trametes gallica* (5 g/L) and using response surface methodology *Pleurotus florida* NCIM 1243 at (15.21 g/L) (Palvannan, & Sathishkumar, 2010). In preference to glucose some other carbon sources also have been screened for increased laccase production, by malt extract in *Phlebia floridensis*, *P. brevispora*, *P. radiata*, and *P. fascicularia* (Da Cunha, *et al.*, 2003); by glucose, fructose, galactose, galacturonic acid, xylose, lactose, sucrose, mannitol, pectin, and inulin in *Botryosphaeria* sp. (D'Souza-Ticlo, *et al.*, 2006). In case of *Trametes versicolor* MTCC 138 using glucose, fructose, lactose, sucrose, glycerol and starch as carbon sources and obtained highest laccase activities (Revankar & Lele, 2006); and mannitol boosted laccase enzymatic activity in strain of *Ganoderma lucidum* 7071-9 and in *Pichia pastoris* till 115.62% (Sun, & coworkers, 2012). *Kluyveromyces* sp. Dwl1 prefers

glucose (10g/L) while *Pichia* sp.Dw2 prefers maltose to yield maximum laccase (Wakil, *et al.*, 2017). In *P. ostreatus*98, the medium with CMC had the highest percent of laccase activity (18 U/g biomass), which was 2 times higher than the medium with sodium gluconate (9.3 U/g biomass) (Mikiashvili, *et al.*, 2006). Laccase production in many fungal strains is suppressed by higher glucose concentrations (Lee, *et al.*, 2004).

In the case of nitrogen source found contradictory observations, in the case of some fungi, laccase production is often triggered by nitrogen depletion (Leatham, & Kirk, 1983) whereas it also found that did not affect laccase activity (Janusz, *et al.*, 2006). Buswell *et al.* (1995) reported a conflicting result in *Phanerochaete chrysosporium* and *Lentinus edodes* strains produced laccase at increased nitrogen levels, and these alterations did not correspond to biomass differences. The optimum concentration of nitrogen requires obtaining the highest laccase production; the level of laccase production is varied with different fungal species due to various concentrations and sources of nitrogen (Eugenio, *et al.*, 2009). Laccase production enhances with higher nitrogen levels (Baldrin, & Gabriel, 2002; Chen, *et al.*, 2003) but certain fungi stimulate the formation of laccase enzyme in nitrogen-limited culture conditions whereas in some of the cases activation of oxidoreductase is generally done in nitrogen-limited medium and inhibited laccase production (Heinzkill, *et al.*, 1998; Lo, & coworkers, 2002; Lee, *et al.*, 2004). *Pleurotus ostreatus* laccase activities were reduced when inorganic nitrogen sources were supplied to a specified medium. The optimum nitrogen source for laccase production by *P. ostreatus* appeared to be peptone, next by casein hydrolysate. However, there is a positive correlation between enzyme accumulation and higher biomass production (Mikiashvili, *et al.*, 2006). Laccase activities were discovered in a medium that was enhanced with by DL-tryptophan in *Grammothele fuligo* (Chauhan, 2019).

The maximum laccase activity of *P. ostreatus* was tested using various nitrogen sources, and it was discovered that malt extract had the greatest laccase activity when compared to yeast extract and tryptone. Wheat bran and malt extract, on the other hand, were both used to help with growth (Nora, & coworkers, 2015). Sodium nitrate (3g/L) is the optimal nitrogen source, for *Pichia* sp.Dw2 and *Kluyveromyces* sp.Dw1 respectively (Wakil, *et al.*, 2017). The best production of laccase was at 1 g NH<sub>4</sub>Cl and 0.5 g malt extract concentration (Sharma, & Arora, 2010). Using a complex nitrogen supply, the greatest laccase activity in *Trametes versicolor* MTCC 138 was achieved (yeast extract) (Strong, 2011). Variety of nitrogen sources provided increased levels of laccase, including potassium nitrate, glycine, beef extract, corn steep liquor, and glutamate at minute quantity (Lee, *et al.*, 2004). Peptone was the most effective source of nitrogen for increasing laccase synthesis (1.8-fold increase) (Strong, 2011). *P. sanguineus* (820 mU) had 2.5 times higher laccase activity than sucrose-asparagine medium, which contains 5 times

as much asparagine (Buswell *et al.* 1995). In *Scytalidium lignicola* laccase synthesis was most aided by sucrose and sodium nitrates as carbon and nitrogen sources (Sidhu, *et al.*, 2017). Among the numerous carbon sources used for laccase production in *Aspergillus* sp. HB RZ4, glucose boosted laccase production by 12.45 times whereas other carbon sources were contributing laccase production in order of glucose> sucrose> starch> maltose> lactose> fructose> glycerol. Yeast extract (6.581 U/mL, an 11.7-fold increase) is an organic nitrogen source and in inorganic NH<sub>4</sub>NO<sub>3</sub> (3.97 U/mL), was obtained maximum laccase activity (Sayyed, *et al.*, 2020).

#### B. pH Effect on Laccase Production

When the ideal pH is acidic (pH 3–5), most laccase production will be excessive. Some applications necessitate a closer match to physiological circumstances, such as pH-7 or even basic medium (Collins, & Dobson, 1995). The ideal pH for laccase synthesis, according to many fungi, is between 5.0 and 6.0 (Papinutti, *et al.*, 2003; Minussi, *et al.*, 2007; Thiruchelvam, & Ramsay, 2007). *Fomes sclerodermeus*, *Kluyveromyces* sp. Dw1, *Pichia* sp. Dw2 and white-rot basidiomycetes, produced maximum titers laccase and biomass on a condition set to pH 6.0 respectively (Wakil *et al.*, 2017). Laccases from *T. versicolor*, *P. cinnabarinus*, *C. gallica*, and *P. ostreatus* are examples of fungal enzymes. Surprisingly, the optimal pH has shifted from 4 to 5, with variety R2 retaining 70% reactivity at pH 6 (Yaver, *et al.*, 1996). The laccase isoforms released by *Trametes pubescens* fungus strain have been found to work best between pH 3.0 and 4.5 (Strong, 2011). In a medium containing glucose and yeast extract, *Aspergillus* sp. HB RZ4 grew under mesoacidophilic shaking conditions, it produced a high amount of laccase. A low pH condition is preferable for fungal laccase production and its functioning (Sayyed, *et al.*, 2020).

#### C. Temperature Effects on Laccase Production

In the organism's development and laccase production, temperature plays a very important role. Results reveal that an average temperature of 25-35°C is best for fungal biomass growth and laccase production, variations occurs due to being incubated in the dark or light (Minussi, & coworkers, 2002). The highest laccase output is reported in *P. ostreatus* prefers temperatures of 25-30°C, while *T. versicolor* prefers temperatures of 35°C (Snajdr & Baldrin, 2007) and *T. harzianum* after 6 days at 35°C and pH 5, (Abd *et al.*, 2016). *Kluyveromyces* sp. Dw1 and *Pichia* sp. Dw2 have suitable temperatures of 30- 35°C, accordingly (Wakil *et al.*, 2017). In this study, three extremely thermostable laccase isoforms with the highest activities were generated in *Steccherinum ochraceum* isolate 1833 in the range 75–80°C (Chernykh, & coworker, 2008; Hilden, 2009). *A. flavus* NG85 showed maximum production of laccase at 36.7°C (Sayyed, *et*

al., 2020). As a result, it's clear that the optimal laccase production varies significantly from one species to next species.

#### D. Effect of Inoculum Time & Size

Cold and pH tolerant *Penicillium pinophilum* strain is already recorded for laccase production up to 35 days of incubation under different cultural conditions, whereas maximum production was recorded on day 28 and steadily decline until day 35 of incubation (Dhakar, & coworker, 2014). *Pleurotus* species on the tenth day of incubation, at temperature and pH 28°C and 5.5 were optimal for laccase production (0.292 U/mL), respectively (Edae, & Alemu, 2017). The greatest laccase activity was discovered to be (43,761.33 ± 3845U/L) by *P. eryngii* on the 20th day (Akpinar, & Urek, 2017) and by *A. flavus* NG85 (8.422 U/mL) on the eighth day of incubation (Sayyed, et al., 2020). The gradual increase in the incubation period resulting in a concomitant rise in laccase amount over the optimal led to a decrease in enzyme production (Ali, et al., 2015).

A lower inoculant quantity is not adequate to initiate proliferation, and a higher inoculant amount inhibits growth (Sabu, et al., 2005). Increased inoculum size reduces laccase production due to rapid nutrient depletion, resulting in lower metabolic activity (Patel, & coworkers, 2009). The best inoculum size for laccase production from *P. martensii* NRC 345 was five discs (Elshafei, et al., 2012). Laccase production employing *Myrothecium gramineum* LCJ177 in solid form was shown to favour highest laccase production (17.56 U/g ds) with an initial inoculum size of 4 g/kg (Daphne, & coworkers, 2019). Results of whole studies show that laccase production increases to an optimal level with gradually increasing inoculum size for various fungal species, and that optimal level occurs after high inoculum levels decline synthesis of laccase.

#### E. Effect of Inducer & Inhibitor on Laccase Production

A significant correlation has been discovered between fungal hyphal branching and expression of laccase (Garzillo, et al., 1998). Various recognition sites specialised for heavy metals and xenobiotics are found in the promoter regions of laccase genes, which trigger laccase synthesis (Robene-Soustrade, & Lung-Escarmant, 1997). The responses of white-rot fungus could cause a wide range of laccase production, and some inducers were also able to produce new isoforms of the enzyme (Eggert, et al., 1996). *Trametes villosa* and *Pleurotus sajor-caju*, on the other hand, have been discovered to contain constitutively expressed laccase genes, which could be linked to a variety of laccase physiological activities in fungi (Marbach, & coworkers, 1985; Da Cunha, et al., 2003), whereas in some cases, a complex cocktail of inducers may be required instead of a single inducer (Grotewold, et al., 1989). Due to inducers uses production suffers from their toxicity, as well as the additional cost, both are negatives.

Copper has been found to be a potent laccase inducer in a variety of species, including *Neurospora crassa* (Dittmer et al., 1997), *Pleurotus sajor-caju* (Sethuraman et al., 1998), *Panus osteratus* (Palmieri et al., 2000), and *Trametes versicolor* (Soden, & Dobson, 2001), *Grifola frondosa* (Arora, & Rampal, 2002), *Trametes trogii* (Levin, & coworkers, 2002), *Phanerochaete chrysosporium* (Chen, & coworkers, 2004), *Lentinula edodes* (Cavallazzi, & coworkers, 2005), *Volvariella volvacea* (Xing et al., 2006), *Paecilomyces* sp. WSH-L07 (Liu, et al., 2009), *Pleurotus florida* NCIM 1243 (Palvannan, & Sathishkumar, 2010), *Shiraia bambusicola* strain GZ11K2 (Du, & coworkers, 2012), *Trametes trogii* TEM H2 (Kocyigit, et al., 2012), *Peniophora* sp. (Shankar, & Shikha, 2012). Copper (Soden, & Dobson, 2001; Baldrian, & Gabriel, 2002) and CuSO<sub>4</sub> (Palmieri, et al., 2000) significantly boost production in *Trametes pubescens*, by acting as a potent inducer of laccase gene transcription in yeast and other fungal species (Akpinar, & Urek, 2017). In place of copper, other metallic ions such as Mg<sup>2+</sup> (Palvannan, & Sathishkumar, 2010), Mo<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup> (Akpinar, & Urek, 2017), enhanced the enzyme activity can also stimulate laccase expression significantly. The optimum production of laccase in *P. feryngii* (43,761.33 ± 3845 U L<sup>-1</sup>) was induced by the inducers Cu<sup>2+</sup> 70 mM, Fe<sup>2+</sup> 18 mM, 0.025 percent (v/v) Tween-80, 4.0 g/L ammonium nitrate 750 μM Mn<sup>2+</sup> (Akpinar, & Urek, 2017).

The addition of xyloidine acting as an inducer had the most fantastic addition to laccase production (Rogalski, et al., 1991; Lu, et al., 1996; Pickeard et al., 1999) in *Trametes villosa*, *T. versicolor*, and *Pleurotus sajor-caju*, (Da Cunha, et al., 2003), *Cariolopsis polyzona* (Jaouani, & coworkers, 2006), *Cariolopsis caperata* RCR2011 (Nandal, & coworkers, 2013). Alcohol, such as ethanol, was used to increase laccase activity in *Pycnoporus cinnabarinus* (Blaich, & Esser, 1975), *Trametes versicolor* (Dhawan, & Kuhad, 2001), and veratryl alcohol in *Peniophora* sp. (Mansur, et al., 1997; Fenice, et al., 2003). As a result, adding cellobiose to some *Trametes* sp. AH28-2 (Dosoretz, et al., 1990), *Pycnoporus* species (Garzillo, et al., 1998) significantly increased laccase activity. Lignin (Gianfreda, & coworkers, 1999) and cycloheximide (Leonowicz, & Trojanowski, 1975) Tween 20 or 80, (Dombrovskaya, & Kostyshin, 1996) and ammonium nitrate (Akpinar, & Urek, 2017) in plenty of fungi, this has resulted in larger production of ligninolytic enzymes. There has been evidence of anionic and cationic surfactants as an effective inducer of laccase from *Pleurotus florida* (Lomascolo, & coworkers, 2003).

By the addition of inducer tannic acid, the laccase gene's expression rises and increases laccase production in the growth medium (Rothschild, & coworkers, 1995). At 1 mM, quantity of EDTA produced considerable laccase secretion. Tested sodium azide at all doses (0.5-2 mM) induced laccase secretion in *Pestalotiopsis* CDBT-F-G1 to isolate colonies (Yadav, et al., 2019). Laccase production was stimulated by Cu<sup>2+</sup> (0.1mM),

tannic acid (0.1mM),  $Mn^{2+}$  (10mM),  $Mg^{2+}$  (10mM), and ethanol (0.5 M) in *Abortiporus biennis* J2 and hindered by  $Ag^{2+}$ ,  $Cu^{2+}$ ,  $Cd^{2+}$ ,  $Fe^{2+}$ ,  $Hg^{2+}$ , and  $Zn^{2+}$  (Liang *et al.*, 2017).

Sodium azide (Sayyed, *et al.*, 2020), SDS (Arora, & Rampal, 2002), and other metal ions, such as  $Al^{3+}$ ,  $As^{2+}$ ,  $Cd^{2+}$ ,  $Co^{2+}$ , and  $Li^{2+}$ , considerably decreased the activity of an enzyme (Baldrian, & Gabriel, 2002), whereas halides (F-> Cl-> Br-> I-),  $Ag^{2+}$ , Hg,  $FeSO_4$ ,  $HgCl_2$ ,  $MnCl_2$ , and  $FeCl_3$  reported as an inhibitor respectively (Bhamare, *et al.*, 2020). Chlorides of metal ions like  $Ba^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Zn^{2+}$ , and other compounds such as L-cysteine, vanillin, ammonium tartrate, gallic acid, urea, PCMB (p-chloromercuribenzoic acid), DDT (Dichlorodiphenyltrichloroethane), and mercaptoethanol do not affect enzyme activity (Arora, & Rampal, 2002). The xyloidine exhibited a diminished effect at greater concentrations, most likely due to toxicity (Lu, *et al.*, 1996).

## VI. MOLECULAR BIOLOGY OF LACCASE

The SDS-PAGE of purified proteins of *Ganoderma lucidum* MDU-7 laccase isozyms' multimeric nature was established. The molecular mass of isozyms was discovered to be between 40 and 66 kDa (Perry, *et al.*, 1993). SDS-PAGE investigation of pure *Aspergillus nidulans* laccase (KF974331) revealed a band with a molecular mass of 66 kDa (Sethuraman, *et al.*, 1998). Laccase from *Trametes* sp. strain AH28-2 has a molecular mass of 62 kDa, according to SDS-PAGE (Hong, & coworkers, 2002). SDS-PAGE examination of *Agaricus bisporus* extracellular laccase protein extracted from compost extract revealed a prominent band with molecular weights of 65 kDa, as well as smaller amounts of polypeptides (More, *et al.*, 2011). The purified laccase of *Pleurotus* sp. SDS-PAGE revealed that it was a monomer with a molecular mass of  $40 \pm 1$  kDa (Ede, & Alemu, 2017). The laccase molecular mass is assessed by SDS-PAGE and is normally in the range of 60 to 90 kDa, coding for 500–600 amino acids in the laccase protein. The research found that about 10–20% of the total molecular weight of laccase protein obtain by glycosylation which causes variation in molecular weight and peptide sequence of laccases. Microbial laccases isoelectric points are generally around 3.6. Numerous fungal genomes have more than one laccase gene (Xiao, *et al.*, 2003). Cultivation conditions extremely correlate with different laccase gene expression levels. For example, when nitrogen concentration enhances in cultivation medium laccase gene transcription is stimulated in the *Pleurotus sajor-caju* and Basidiomycete I-62 (CECT 20197) (Patel, & coworkers, 2009). Laccase closely resembles the ascorbate oxidase subunit and has shown significant homology with the mammalian plasma ceruloplasmin (Gernann, & Lerch, 1986).

## VII. ISOZYMES OF LACCASE

Extracellular laccases in white-rot fungus appear in isozyms that may be inducible or intrinsic. Basidiomycetes, including

*A. bisporus* (Wood, 1980), *P. ostreatus* (Sannia & coworkers, 1986), *T. versicolor* (Rogalski, *et al.*, 1991), *C. cinereus* (Schneider, & coworkers, 1999), and *P. chrysosporium* (Rodriguez, *et al.*) were able to produce several isozyms. Various laccase isoforms were produced by *Coprinus plicatilis*, *Fomes fomentarius*, *Heterobasidion annosum*, *Hypholoma fasciculare*, *Kuehneromyces mutabilis*, *Leptoporus litschaueri*, *Panus stipticus*, *Phellinus igniarius*, *Pleurotus corticatus*, *Polyporus brumalis*, *Stereum hirsutum*, *Trametes gibbosa*, and *T. hirsuta* (Baldrian, 2006). In liquid culture, the *Ganoderma lucidum* (Ko, & coworkers, 2001) and *Pleurotus pulmonarius* (De Souza, *et al.*, 2004) produced more than three laccase isozyms, and the basidiomycete CECT 20197 produced seven laccase isozyms (Mansur, *et al.*, 1997), *Marasmius quercophilus* strain-17 produced four stimulated isozyms and three conservative isozyms (Farnet, *et al.*, 2000). *Pleurotus* sp. has nine constitutive laccase isozyms, six of which have been extracted and identified (Liu, *et al.*, 2000). A report by Courty, *et al.*, (2008) showed that 17 different isolates of fungus after induction with different aromatic compounds were consistent similar isoenzyme arrangement (one of the three laccase bands on SDS PAGE). Isozyme patterns have also been discovered in ectomycorrhiza, Deuteromycetes, and basidiomycetes, among other fungi (Zervakis, & coworkers, 2001; Tanesaka, 2012).

## VIII. ROLE OF LACCASES IN EFFLUENT TREATMENT

Today, view that xenobiotic compounds are created a high level of pollution in the world by a chemical which is a dominant cancer and mutation causing elements. Classical treatment techniques are quite expensive and utilise a lot of chemicals and energy, whereas biological treatment appears to be a good option. As a result, using enzymes to bioremediate contaminants is considered an environmentally acceptable, economical, and effective process. The laccases enzyme-based treatment processes are especially interesting because fewer induction conditions are required for laccase production than Lignin peroxidase and Manganese peroxidase (Bettin, *et al.*, 2011). Laccase has shown great potential for the treatment of wastewaters decolorization and detoxifications of effluents discharge from industries (Ikehata *et al.*, 2004; Chandra and Chowdhary, 2015) such as pulp and paper industry (Archibald *et al.*, 1997), winery/alcohol distilleries (Fitzgibbon *et al.*, 1998, Yague *et al.*, 2000), food and beverage industry (Minussi *et al.*, 2002), olive milling (Jaouani *et al.*, 2003) and textile-industry wastewaters containing dyes (Wesenberg *et al.*, 2003).

Presently laccases are applied as a green remedy to treat organochloride of phenol, polynuclear aromatic hydrocarbons, complex organic polymers structure of lignin, compounds of organophosphorus (OPs) are a diverse group of chemicals, including insecticides, benzenol and its derivatives, and colour compounds bound chemically mean dyes (Murugesan *et al.*, 2007; Saratale *et al.*, 2011; Khan *et al.*, 2013; Viswanath

*et al.*, 2014). Laccases can also convert 1, 1'-(2, 2, 2-Trichloroethane-1, 1-diyl) bis (4-chlorobenzene) means (DDT) (Zhao *et al.*, 2010) and 2, 4-DCP a chlorinated derivative of phenol (Bhattacharya *et al.*, 2009) in soil. Researchers reported that laccase produced in greater concentrations during the idiophase (Collins and Dobson, 1997).

Due to their high water solubility and poor biodegradation, textile dyes are by far the most troublesome chemicals found in textile effluents (Khelifi, *et al.*, 2009). Textile dyes considerably reduce the quality of aquatic environment as a result of untreated effluent discharge (Bhatia, 2017). Increases in relatively high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Setiadi, & coworkers 2006) contribute to a decrease in the photosynthesis rate (Imran *et al.*, 2015) and dissolved oxygen concentration influencing the natural aquatic biotic life system (Hassan & Carr, 2018), entering the food chain, providing recalcitrance and biomagnification (Sandhya, 2010), and potentially causing cytotoxicity, mutation, and various types of cancer (Aquino *et al.*, 2014, Khatri *et al.*, 2018). In agriculture, wastewater is frequently used for irrigation in developing countries (Rehman *et al.*, 2018). Azo compounds dye

has a deleterious impact on soil microbial populations as well as plant development and reproduction (Imran *et al.*, 2015).

Dye wastewater treatment methods that have been used in the past unproductive and uneconomical. Consequently, laccases based processes appear to be an appealing alternative along with its ability as eco-friendly diverse degrading dyes biocatalysts (Yang, & Yu, 1996; Kalsoom, & coworkers, 2015). The decolorization of dyes by using aerobic bacteria with limited ability to target a specific chemical structure and anaerobic bacteria produced amines that are potentially mutagenic and/or carcinogenic (Wong, & Yu, 1999). Bacterial laccases actions are less specific to the substrate (Thurston, 1994; Wong, 2009). The action of fungal laccase on chromophore molecules such as polymeric dyes, remazol brilliant blue R, triarylmethane, triphenylmethane azo, anthraquinone, heterocyclic, and indigoid dyes has been examined (Abadulla *et al.*, 2000). The black liquor cause altered chemical oxygen demand (COD), biochemical oxygen demand (BOD), and chlorophenol content released into water bodies, as well as raised colour and pH, had a severe influence on the ecosystem (Claudia, *et al.*, 2018).

Table I. The use of certain fascinating fungal laccases which decolorize and destroy dyes in waste waters

Fungal laccases	Decolorized and degraded Dye	References
<i>Pycnoporus sanguineus</i>	Triphenylmethane dyes (bromophenol blue and malachite green)	Rodriguez, & coworkers, 1999
<i>Flavodon flavus</i>	Azure B and Brilliant Blue R	Abadulla <i>et al.</i> , 2000
<i>Thelephora</i> sp.	Azo dyes- Orange G and Congo Red	Selvam, <i>et al.</i> , 2003
<i>Trametes hirsuta</i>	Triarylmethane, indigoid, azo, and athraquinonic -textile dyes and 23 industrial dyes Bromophenol Blue and Indigo Carmine	Setti, <i>et al.</i> , 1999 Rodriguez, & coworkers, 2005; Rodriguez Couto <i>et al.</i> 2004
<i>Trametes versicolor</i>	Degradation dye brilliant green1 and acid green 16 Amaranth, Tropaeolin O, Reactive Blue 15, Congo Red, and Reactive Black 5 with no dye sorption; partially decolorizes Brilliant Red 3G-P, Brilliant Yellow 3B-A, and Remazol Brilliant Blue R with some dye sorption	Casas <i>et al.</i> , 2007
<i>Trametes trogii</i> BAFC 463	85% of Indigo carmine, xylydine, Malachite green, Gentian violet, Bromophenol blue, 65% of fast blue RR, and 30% of Azure B and Methylene Blue	Blanquez & coworkers, 2004; Grassi, <i>et al.</i> , 2011
<i>Marasmius</i> sp.	Violet, red, orange, and yellow dyes, blue dyes	Schückel <i>et al.</i> , 2011
<i>Pleurotus ostreatus</i> URM 4809	86% of the anthraquinone dye remazol brilliant blue R	Simoes, <i>et al.</i> , 2019
<i>Ganoderma lucidum</i> E47	Xanthene, azo and triarylmethane dyes organic dyes: Bengal rose; blue black naphthol; congo red; methyl orange; bromo- cresol green; bromo- cresol purple; bromo- phenol blue; and phenol red,	Palazzolo, & coworkers, 2019
<i>Oudemansiella canarii</i>	Congo red	Iark, <i>et al.</i> , 2019
<i>Pleurotus sajor-caju</i>	Acid Blue 80, Acid Green 28, Brilliant Green, Bromocresol Green, Coomassie Brilliant Blue G-250, Disperse Blue 79, Reactive Red 198, Reactive Blue 220, Congo Red, Gentian Violet, Malachite Green, Methyl Violet, Disperse Red 324, Levafix Brilliant Red E-4BA, Acid Red 315, Disperse Orange 30	Fernanda Bettin, <i>et al.</i> , 2019
<i>Aspergillus</i> sp. HB_RZ4	88% Bromothymol blue, 96% Bromocresol purple, and 99% Bromophenol blue dyes	Sayyed, <i>et al.</i> , 2020

Genetically engineered fungal laccases for dyes degradation and decolorization.		
<i>Phanerochaete chrysosporium</i> , <i>T. trogii</i> , <i>Pleurotus ostreatus</i> , and <i>T. Versicolor</i>	Amaranth, Remazol Black B, Remazol Orange, Remazol Brilliant Blue, Reactive Blue, and Tropaeolin O	Swamy & Ramsay, 1999
Recombinant lcc1 gene from <i>Trametes trogii</i> in <i>Pichia pastoris</i> F	Amaranth, Carmoisine, Cochineal red, Sunset yellow, Patented blue, Blue indigo, and Alizarin red	Colao, <i>et al.</i> , 2006
<i>Aspergillus</i> expressing a laccase from <i>Myceliophthora thermophila</i>	Anthraquinonic dyes-Acid blue 25 and Acid green 27	Kunamneni, <i>et al.</i> , 2008
Recombinant LCC3 from <i>Trametes trogii</i> BAFC 463 in <i>Pichia pastoris</i>	50–100% decolorizing ability of azoic, indigoid, triarylmethane, and anthraquinonic	Campos, & coworkers, 2016
Recombinant laccase (Lcc IIIb) from <i>Trametes versicolor</i> expressed in <i>Yarrowia lipolytica</i> C	43% Bromocresol purple, 54% Safranin, 55% Malachite green, 49% Crystal violet, 56% Bromothymol blue, 53% Nigrosine, and 37% Phenol red	Darvishi, <i>et al.</i> , 2018
<i>P. pastoris</i> or <i>A. thaliana</i> expressing Lcc9 from <i>Laccaria bicolor</i>	Crystal violet	Wang, <i>et al.</i> , 2018

Table II. Some unique fungal laccases are being used in various industrial effluent treatments by removal of phenol and phenolic compounds, Chemical Oxygen Demand & decolorization.

Fungal Laccases	Effluent treatments	References
<i>Clavariopsis gallica</i>	Degrade xenoestrogen nonylphenol	Calvo, <i>et al.</i> , 1998
<i>Trametes</i> sp.	Bioremediates the distillery wastewater generated from the sugarcane molasses with high content of organic matter	Gonzalez, <i>et al.</i> , 2000
<i>Trametes villosa</i>	Degrades bisphenol	Raghukumar, 2000
<i>Flavodon flavus</i>	Decolourize the effluent from a Kraft paper mill bleach plant	Soares, & coworkers, 2001
<i>Corioliopsis rigida</i> LPSC 232	Detoxification of water-soluble fraction	Torres, & coworkers, 2003
<i>Pleurotus ostreatus</i>	Degradation of PCBs as well as phenol	Keum and Li, 2004
<i>Trametes pubescens</i> MB 89	79±1.1% COD removal, 80±4.6% total phenols removal, 71 ± 1.6% decrease in colour from wine distillery wastewater	Strong and Burgess, 2007
<i>Trametes pubescens</i>	Biodegradation of a mixture of pentachlorophenol (9PCP), 2 chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), and 2, 4, 6-trichlorophenol (2,4,6-TCP)	Gaitan <i>et al.</i> , 2011
<i>Pleurotus sajor-caju</i>	Oxidize catechols, quinols, methoxyphenols, some aromatic amines and their methyl derivatives, and also resorcinol and phloroglucinol	Zucca <i>et al.</i> , 2011
<i>Trametes versicolor</i>	degradation of PCBs as well as phenol Colour and aromatic compounds were reduced up to 70–80% and COD was reduced up to 60%. Bisphenol A	Keum and Li, 2004 Blázquez & coworkers, 2004 Margot, <i>et al.</i> , 2013
<i>Pleurotus ostreatus</i> and <i>Pichia pastoris</i> (which produces rPOXA 1B laccase)	80.3% COD removal and 70.6% decolourization from Synthetic Black Liquor	Claudia, <i>et al.</i> , 2018

Fungal laccases, take attentiveness in the field of biotechnology because they have the capability to oxidized substrates due to high redox potential ( $E^\circ > 400$  mV) (Rodgers, *et al.*, 2010). Synthetic dyes, which are hazardous to mammals, are found in textile and paper industry effluents. As a result, efforts to eradicate industrial wastewaters employing laccases from *Trametes versicolor* and *Trametes trogii* “Table I” have been made (Grassi, *et al.*, 2011; Garcia, *et al.*, 2019). Laccases from white-rot fungus, such as *T. versicolor*, immobilised onto silica-alginate and loofa-sponge as support for phenol removal, have the potential to degrade phenolic compounds (Carabajal, *et al.*, 2016). *Trametes pubescens* crude extract was employed in the study of degradation of chlorophenols “Table II” (Gaitan *et al.*, 2011), whereas purified laccase from *Trametes villosa* able

to degrade BPA without any mediators (Piscitelli, *et al.*, 2010). *Pycnoporus sanguineus* (CS43) immobilised on ceramic membranes that decompose BPA (20 mg/L) within 24 hours. “Table II”. *T. pubescens* MB was able to eliminate 8979.1% COD, 80±4.6% total phenolic content, and reduce 71.6% in colour from wine distillery wastewater (Strong & Burgess, 2007) “Table II”.

To find out the interest of laccase for the industrial need is challenging, especially when the enzyme isn't widely expressed as a result, heterologous laccase expression can be used as a substitute (Piscitelli, *et al.*, 2010; Viswanath, *et al.*, 2014). Many fungal laccases, such as *Pichia pastoris* and *Yarrowia lipolytica*, in filamentous fungi *Aspergillus oryzae*, *A. niger*, *T. villosa* (Yaver, *et al.*, 1996; Bohlin, *et al.*, 2006), and *Pycnoporus*



*cinnabarinus* (Madzak, *et al.*, 2005) have been found to have heterologous expression. Yeasts and filamentous fungi as a host are typically more appealing for heterologous protein production to produce large proteins amount into the growing medium, faster microbial growth, easy gene manipulation, as well as the potential to produce posttranslational modifications. Yeasts and filamentous fungi that serve as hosts are frequently more appealing (Piscitelli, *et al.*, 2010).

Bioremediation has also benefited from the use of recombinant fungal laccases. Several polluting dyes, has been reported such as indigo carmine, etc. undergo oxidative degradation, by recombinant proteins Lcc1 and Lcc3 isolated from *T. troglitii*, produced in *P. pastoris* (Colao, *et al.*, 2006; Campos & coworkers, 2016). After 4 hours of treatment, a recombinant laccase (Lcc IIIb) from *T. versicolor* in *Y. lipolytica* decolorizes five phenolic azo dyes with > 40% efficiency (Darvishi, *et al.*, 2018). Likewise, *Laccaria bicolor*, an ectomycorrhizal fungus found in *P. pastoris* and *A. thaliana*, was established as a means of decolorizing > 80% of the crystal violet dye (Wang, *et al.*, 2018). In another study, *Pleurotus ostreatus* and *Pichia pastoris* (producers of rPOXA 1B laccase) were shown 80.3% COD removal and 70.6% decolorization from Synthetic Black Liquor (Claudia, *et al.*, 2018) "Table II".

#### CONCLUSION

Presently, the diversity of pollutants discharged without any treatment into water bodies by various industries causes' water pollution and health issues. There is an obvious need to overthrow contamination of the world's water sources. Laccases, an efficient biocatalytic tool, are now being studied as an alternate approach for oxidising these molecules, with the goal of producing fewer toxic and hazardous inactive chemicals while also removing poisonous xenobiotics from the environment. Their wide substrate specificity and usability drew interest in research as well as in commercial processes such as pulp delignification, textile bleaching, phenolic elimination, and biosensors. This review compiles recent laccase findings from a variety of sources, characteristics, mechanisms of action, and optimization of various fungal laccase production conditions and enzymatic activity to achieve their efficient use in pollutant molecule bioremediation and biotransformation in water. Consequently, there has been a need to discover and innovate new laccases capable of catalytic activity under adverse conditions, as well as develop novel approaches and techniques to facilitate their widespread use in the treatment of pollutants from water. Laccase has found applications in the design of biosensors and nanotechnology.

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