

Dye Decolourization using Fungal Laccase: A Review

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Abstract- Laccases are multi-copper oxidases, found in many plants, fungi and microorganisms. It was found that laccase plays a crucial role in the formation of lignin by promoting the oxidative coupling of monolignols, a family of naturally occurring phenols. The isolation and identification of laccase from white rot fungi plays a vital role in exploiting its environmental and biotechnological application. The increased use of a wide varieties of synthetic dyes in different industries has raised a hazardous environmental aware. Researchers found that 17-20% of fresh water pollutions are caused by the presence of these dyes. Dyes are the most difficult constituents to remove by conventional waste water treatment, and are mainly removed by physical and chemical methods, which are expensive. Hence the biological processes become the most prominent technology for dye decolourisation. Laccase based decolourization treatment are highly advantageous to bioremediation technologies. This paper reviews the ability of laccase to decolourize dyes in different industries.

Keywords – Laccase, Decolourization, White rot fungus, Waste water treatment, Lignin

I. INTRODUCTION

Synthetic dyes are broadly used in textile dyeing, paper printing, color photography, pharmaceutical, food, leather industries and cosmetics. Annual production of synthetic dyes has exceeded over 7×10^5 metric tons [1]. Synthetic dyes used are toxic, mutagenic and carcinogenic. Most of the dyes are complex aromatic structure allows for their resistance to light and water oxidation and biodegradation, which is the feature desired by industry using the dye, but it is dangerous for the environment. Coloured waste water is mainly eliminated by physical and chemical processes such as coagulation, flocculation, adsorption, precipitation and membrane separation. However they are very expensive and have drawbacks [2], such as low efficiency, limited versatility, interference by other waste water constituents and handling of the waste generated. Microbial decolourisation is a cost effective process which is receiving much attention for the treatment of dye-containing waste water [3].

Fungi could be an excellent candidate for dye removal [2], can exploit marginal living conditions in large part because they produce unusual enzymes capable of performing chemically difficult reactions [4], and also possess the following advantages over single cell organisms: solubilization of the insoluble substrate and tolerance for high concentrations of the toxicants, as well as greater physical and enzymatic contact with environment because of an increased cell to surface ratio. [3].

Laccase belongs to a group of copper-containing polyphenol oxidases that can catalyze the four-electron reduction of O_2 to H_2O coupled with the oxidation of phenolic compounds. As a ligninolytic enzyme produced by white-rot fungi, laccase exhibits a broad substrate specificity and unique ability of biodegradation [5]. Laccases can be used for the treatment of effluents from pulp mills or from other industries containing chlorolignins or phenolic compounds.

The main objective of this paper is to summarize the means of information available in literature with regard to laccase isolation, identification and mechanism and its role in the decolourisation of different industrial effluents. The present review, therefore should help to elucidate the mechanism of laccase in dye decolourization.

II. SOURCES OF LACCASE

Yoshida in 1883 first described laccase when he extracted it from the Japanese lacquer tree, *Rhusvemicifera vermicifera*. Laccase was demonstrated to be present in fungi for the first time in 1896 by both Bertrand and laborde. Laccase are widely distributed in higher plants, bacteria, fungi and insects [6, 7] which are described as follows:

Plant: Lacquer, peach, mung bean, mango, cabbages, turnips, beets, apples, asparagus, potato and various other vegetables has been detected for the presence of laccase enzyme.

Fungi: Fungal laccases have higher redox potential than bacterial or plant. Fungi from deuteromycetes, ascomycetes as well as basidiomycetes are the good producers of laccase.

Bacteria: Bacterial laccase was first reported in *Azospirillum lipoferum* [8]. Nowadays laccase is found in many bacteria including *Bacillus subtilis*, *Bordetella compestris*, *Escherichia coli*, *Pseudomonas syringae*.

Insects: Different insects have also been reported for the production of laccase. Some of them are Bombyx, Calliphora, Diptoptera.

III. ISOLATION OF LACCASE

Laccases are glycosylated proteins and have been isolated and purified from fungi, bacteria, plant, insects and lichens. The variety of commercial and industrial application of laccases has attracted much attention towards the research addressing different aspects of the protein characterization, production and fit for purpose molecule [9].

Laccases can be isolated from soil samples and tree bark scrapings by employing standard serial dilution method. They can be screened based on the growth on potato dextrose agar media containing specific substrate which included ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), guaiacol, tannic acid, syringaldehyde [10].

IV. PURIFICATION OF LACCASE

Ammonium sulphate is being commonly used for the enzyme purification for many years. Recently researchers have found much more efficient methodologies such protein precipitation by ammonium sulphate, anion exchange chromatography, desalt/buffer exchange of protein and gel filtration chromatography. Previously a lignolytic MnP from *Phanerochaete sp* was salted out at 65% (NH₄)₂SO₄ saturation with 2.68-fold purification and 5.56% yield.

The purification step for laccase from *Schyzophyllum commune* are given in Table 1 [11]. A single peak obtained in gel filtration profile (figure:1) showed that laccase had a single enzyme activity and there was no isozyme of laccase [11].

Sr. No	Purification steps	Total volume (mL)	Total enzyme activity (U)	Total protein content (mg)	Specific activity (U/mg)	Yield (%)	Purification (fold)
1	Crude laccase	500	183500	4535	40	100	1
2	(NH ₄) ₂ SO ₄ ppt	40	88750	855	104	48	2.6
3	Dialysis	40	79166	718	110	43	2.75
4	Sephadex G100	12	6031	38	158	3	3.95

Table 1. Purification summary of laccase from *Schyzophyllum commune* IBL-06

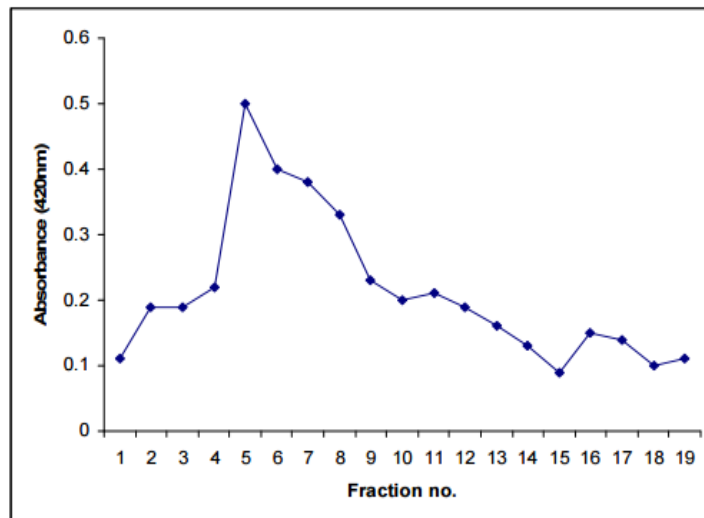


Figure 1. Gel filtration chromatography of laccase produced by *Schizophyllum commune* IBL-06

V. MECHANISM OF LACCASES

The catalysis of laccase occurs with reduction of one molecule of oxygen to water accompanied with the oxidation of one electron with wide range of aromatic compounds which include polyphenols, aromatic amines, methoxy-substituted monophenols (figure 2). This oxidation results in generation of oxygen centered free radicals that can be converted to quinone in a second enzyme catalysed reaction. Laccase catalysis occurs in three steps: (1) type I Cu reduction by substrate; (2) electron transport from type I Cu to type II Cu and type III Cu trinuclear cluster. These three types can be distinguished by using visible electronic paramagnetic resonance (EPR) spectroscopy. Type I Cu gives blue colour to protein at absorbance of 610 nm which is EPR detectable. Type II Cu doesn't give colour which is EPR detectable. Type III Cu gives weak absorbance in near UV region but not detected by EPR signal [12].

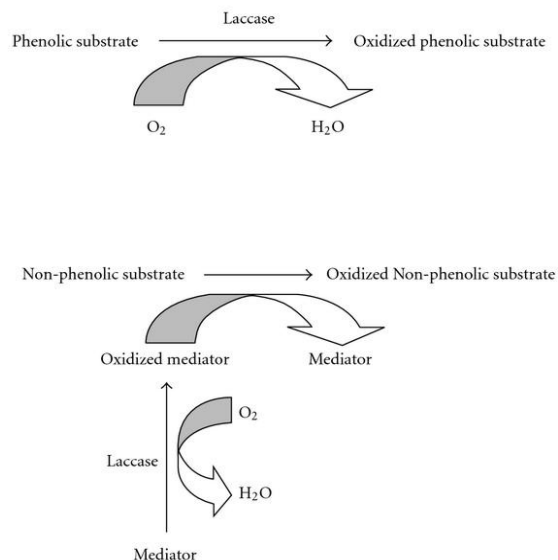


Figure 2. Mechanism of laccase action for both phenolic and non-phenolic substrate

VI. TYPES OF CULTIVATION

Enzyme production is a high-ranking field of biotechnology. Most enzyme manufacturers produce enzyme by submerged fermentation (SMF) techniques. Over the past decades there has been an increasing drift towards the use of solid state fermentation technique [13]. However laccases have been produced intensely in both submerged and solid state modes of fermentation. Wild type filamentous fungi are used for large scale production of laccase in different cultivation techniques. Table: 2 list the different cultivation techniques that have been adopted for large-scale production of laccase using wild-type filamentous fungi [12].

Fungi	Type of cultivation	Inducer	Laccase Activity (U/L)
<i>Pycnoporus cinnabarinus</i>	Submerged	10 mM Veratryl alcohol (VA)	280
<i>Trametes pubescens</i>	Submerged	2 mM Cu ²⁺	333,000
<i>Neurospora crassa</i>	Submerged	1 μM cyclohexamide	10,000
<i>T. versicolor</i>	SSF (Immersion, nylon sponge)	Tween 80	229
<i>T. versicolor</i>	SSF (Immersion, barley bran)	Tween 80	600
<i>T. versicolor</i>	SSF (Expanded bed, nylon sponge)	Tween 80	126
<i>T. versicolor</i>	SSF (Expanded bed, barley bran)	Tween 80	600
<i>T. versicolor</i>	SSF (Tray, nylon sponge)	Tween 80	343
<i>T. versicolor</i>	SSF (Tray, barley bran)	Tween 80	3500
<i>T. hirsuta</i>	SSF (Tray, grape seeds)	—	18,715

Table 2: Production of laccase in different cultivation modes

VII. DYE DECOLOURISATION

More than 10000 various dyes stable to light, chemicals and microbial degradation are manufactured and used by textile, cosmetic, plastic and printing industries. Based on the chemical structure of chromogenic groups, dyes are classified as azo, heterocyclic/polymeric or triphenyl methanes and about 60% of produced dyes belongs to the azo groups which are categorized as mono-azo, diazo and triazo dyes [14]. Small scale industries in India contribute 3900 million litres waste water per day. Presence of very low concentrations of dyes in effluents is highly visible and undesirable [15]. Furthermore they damage the esthetic nature of water and reduce light penetration through the water's surface and the photosynthetic activity of aquatic lives. Decolourisation by conventional waste water treatment is inefficient since they are costly, time consuming and often methodologically demanding.

At present a number of studies are focused on white rot fungi which have the capability to degrade various xenobiotic compounds including dyes since they produce some extracellular lignolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase. Laccase have been received a great attention due to their efficiency and low cost of degradation of the organic pollutants including synthetic dyes [16].

Recent research found that laccase of *Paraconiothyrium variable* was the most efficient enzyme with 72.2% removal of bromophenol blue [16]. It was found that this can be achieved after 30 minute treatment in absence of hydroxybezotriazole (HBT). However, decolourisation using laccase of *Aspergillus oryzae* and *Trametes versicolor* did not increase higher than 25.3% even after 3 hours incubation. Compare to laccases from *Trametes versicolor* and *Aspergillus oryzae* which represented 30.3% and 13.3% decolourisation of comassie brilliant blue [14].

Research by Vaidyanathan *et al.* [16] shows that the individual dye structures influence the decolourisation extent obtained by laccase, indicating the specificity of laccase towards different dye structures. This reveals the high potential of laccase from *Pleurotus ostreatus* for dye decolourisation.

Darshan and Kirti studied on decolourisation of reactive orange 16 by *Comamonas acidovorans* shows that physico chemical parameters such as temperature, pH, culture condition, co-substrate, nitrogen source, salt concentration and metal ions has significant influence on dye removal efficiency. Isolate has shown maximum decolourization at 37⁰C, pH 6.85 using lactose best electron donor and yeast extract as nitrogen source [17].

The purified laccase of *Paraconiothyrium variable* studied for its capacity in the removal of aromatic compounds was immobilized on porous silica beads and applied for decolourization of two synthetic dyes of acid blue 25 and acid orange 7 and was found that pH and thermal stability of immobilized enzyme was improved compared to free laccase, both applied dyes were efficiently decolourised using immobilized laccase [18].

A work on decolourisation of an azo dye in an aqueous solution by ozonation in a semi batch bubble column reactor were evaluated and found to be an efficient technique for waste water treatment. The colour removal efficiency of over 98% was obtained at contact time of 30 min, which proved that ozonation is a very efficient process for colour removal [19].

Robert Mass *et al* studied the decolourisation of azo dye RR2 in semi continuous anaerobic reactors and found that after an initial adsorption of dye on the biomass subsequent degradation of dye occurred which suggest that the primary mechanism of dye removal appears to be adsorption of dye on biomass followed by biodegradation [20]. White rot fungi were capable of secreting active ligninolytic enzymes and decolourising the pulp and paper mill waste water. On direct addition of glucose to the waste water source, it has been found to be more economical to reduce or substantially remove color from the waste water by additionally adding white rot fungi in the presence of cellulose and hemicellulose [21].

Decolorization of pulp and paper mill effluent by lignin-degrading fungus *Aspergillus flavus* strain F10 indicate that the utilization of *Aspergillus flavus* F10 is suitable for the decolorization and polyphenolic groups removal from soda pulping effluents in the presence of a carbon source and the toxicity of the treated effluent was found to be reduced [22].

Poonam Dayaram *et al* observed that the ready availability of laccase from *Polyporus rubidus* and its robust characteristics help to overcome the barriers for waste water treatment. Their results suggested that laccase from *Polyporus rubidus* could decolourise synthetic dyes in broad range of concentration without the need for redox mediators, provided that the dyes are water-soluble. Also found out that immobilized enzymes tend to have higher activity and are more resilient to environmental perturbations such as pH or exposure to toxic chemical concentration than suspension cultures. The enzyme from *Polyporus rubidus* was also found to be effective in removing colour from untreated effluents. Through this study they showed that white rot fungi, *Polyporus rubidus* is far more efficient for decolorizing a range of industrially important textile dyes and real waste water from industries [23].

VIII. CONCLUSION

Colour is all around us. Wherever we look we see colours, even if we do not always notice them. Nowadays the human activities are disturbing these colours by disposing synthetic colour in nature. Synthetic dyes are broadly used in textile dyeing, paper printing, color photography, pharmaceutical, food, leather industries and cosmetics. Most of these industries dispose their effluent without any treatment technologies. Dyes are the most difficult constituents to remove by conventional waste water treatment, and are mainly removed by physical and chemical methods, which are expensive. Hence the biological processes become the most prominent technology for dye decolourisation. Fungi could be an excellent candidate for dye removal. Laccases are multicopper oxidases, found in many plants, fungi and microorganisms. It was found that laccase plays a crucial role in the formation of lignin by promoting the oxidative coupling of monolignols, a family of naturally occurring phenols. Both water consumption and effluent toxicity in dyeing industries could be reduced by enzyme remediation with laccases. Hence laccase is receiving much attention of researchers around the globe.

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