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A Review on *Pycnoporus sanguineus* Based Biotechnological Approaches for Bioremediation of Industrial Waste

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ABSTRACT

The diverse uses of white rot fungi and their enzymes are becoming more and more popular nowadays in the decolorization of dyes, wastewater treatment and bleaching in paper industry. In this context, *Pycnoporus sanguineus* a polyporoid white rot species presents a promising solution due to the secretion of the ligninolytic enzymes such as laccases and peroxidases. These enzymes exhibit broad substrate specificity and are effective in degrading synthetic dyes and other persistent organic pollutants. The polyporoid fungus is particularly noted for its capability to decolorize and detoxify wastewater from industries such as textiles, leather, and paper, offering an eco-friendly alternative for wastewater treatment. The present review summarizes the uses of *Pycnoporus sanguineus* in the decolorization of dye, wastewater treatment, biodegradation and bleaching in paper industries.

Keywords: *Pycnoporus sanguineus*, Pharmaceutical waste water treatment, Dye decolorization, Environmental remediation

INTRODUCTION

The growing interest in environmental issues, particularly water, has given rise to a new industrial constraint: reduce the toxicity and colour of wastewater. Many dyes are toxic in addition to their detrimental effects on chemical oxygen demand and visual effect. These dyes even in very small quantities, can negatively affect aquatic life due to the reduced light diffusion (Clarke and Anliker, 1980; DeVito, 1993; Banat *et al.*, 1996). According to current studies, white rot fungi appear to be a potential approach to decrease this pollution as they secrete ligninolytic enzymes such as benzenediol, oxygen oxidoreductase, p-diphenol oxidase, urishiol oxidase and other laccases. These enzymes have a low specificity for substituted aromatic structures (Abadulla *et al.*, 2000; Hess, 2002; Nilsson *et al.*, 2006).

Pycnoporus sanguineus (L.) Murrill, a wood inhibiting white rot Agaricomycetous polypore, is slow growing macrofungi found in tropical and subtropical regions. Basidiocarp is bright reddish orange in colour, annual, smooth, flabelliform, surface velutinous to glabrous, leathery, coriaceous to corky consistency with acute margin. Size of basidiocarp is generally 30–30 × 22–30 × 5– 10 mm (diam., width and thickness) (**Figure 1**). Trimitic hyphal system, spores are cylindrical with 5–6 × 2–3 µm in size, smooth, hyaline, non-amyloid and thin-walled. Hymenophore is poroid, reddish orange in colour, 4-6 pores per mm, circular tubes concolor to hymenophore (Téllez-Téllez *et al.*, 2016).

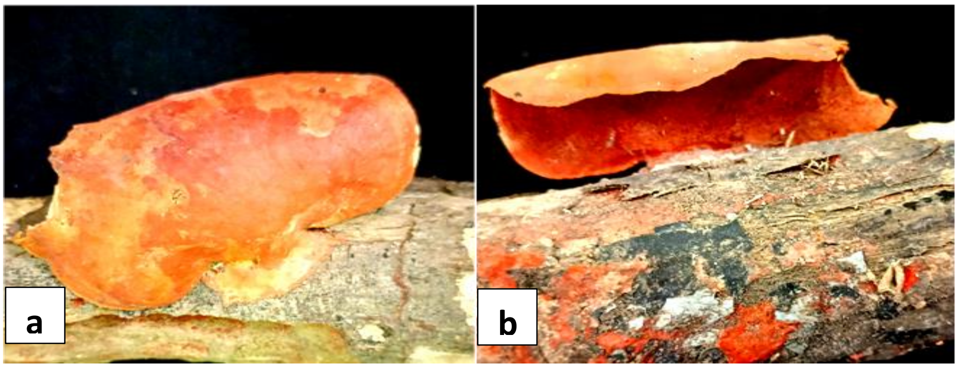

Figure 1: a, Pileus on rotten wood; b, Hymenophore

Pycnoporus sanguineus has garnered attention due to its versatile applications in the field of environmental biotechnology. Various pollutants from paper and dye industries (Vanhulle *et al.*, 2007), agricultural waste (dos Santos *et al.*, 2023) have devastating effects for the ecosystem. The notable enzymatic capabilities of *Pycnoporus sanguineus*, specifically production of ligninolytic enzymes such as laccases, peroxidises, and lignin peroxidises, plays a pivotal role in the degradation and transformation of a wide range of organic pollutants, including synthetic dyes and other recalcitrant compounds found in wastewater from textile, leather and paper industries. *Pycnoporus sanguineus* is also recognized for its ability to decolorize and detoxify synthetic dyes, a crucial capability in industries dealing with textile effluents and dye-contaminated wastewaters as well as pharmaceutical wastewater. The fungus's ability to break down and metabolize complex dye compounds determines its effectiveness in dye decolorization and provides an environmentally friendly way to clean up the environment. Thus, *P. sanguineus* holds significant potential as a bioresource for eco-friendly applications in the remediation of dye-polluted environments (Lomascolo *et al*., 2011).

INDUSTRIAL APPLICATIONS OF *PYCNOPORUS SANGUINEUS*

Pycnoporus sanguineus is utilized in various industrial applications due to its ability to produce laccases and peroxidases which are effective in dye decolorization, wastewater treatment, and the degradation of lignocellulosic materials. These capabilities make it an important bioresource for eco-friendly and sustainable processes in industries such as textiles, paper, and pharmaceuticals**.**

Role in dye decolorization

The widespread use of chemical dyes in textiles industries has led to their release into the environment, increasing Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and water toxicity due to heavy metal contamination. Conventional treatment methods are costly and energy-intensive, making microbial bioremediation a promising and innovative solution for the biodegradation and detoxification of these hazardous dyes (Das *et al*., 2023). Dye decolorization is an important process in environmental bioremediation, particularly for the treatment of textile industry wastewater that contains synthetic dyes. *Pycnoporus sanguineus* has been studied for its ability to decolorize or degrade various types of dyes. The ligninolytic enzymes secreted by this blood-red bracket fungus possesses the capability to degrade a wide range of organic compounds, including dyes. These enzymes are involved in the breakdown of complex structures found in dyes, resulting in their decolorization and degradation. Several research studies have investigated the decolorization potential of *P. sanguineus* for different dyes, such as azo dyes, anthraquinone dyes and triphenylmethane dyes (Pointing *et al.*, 2000). The fungus has been shown to effectively decolorize various dyes under different conditions, including different dye concentrations, pH levels, and incubation. The laccase enzyme produced by the submerged liquid culture of *P. sanguineus* has the capacity to decolorize of 2 azo dyes and complete decolorization of 2 triphenylmethane dyes, bromophenol blue and malachite green. These study suggested that *P. sanguineus* was suitable to treat dye-containing wastewaters (Pointing *et al.*, 2000; Lomascolo *et al*., 2011). Similarly, anthraquinone dye remazol brilliant blue R (RBBR) was decolourized after incubation at 40°C with laccase of *P. sanguineus* without additional redox mediator (Lu *et al.*, 2007). The discovery of natural mediators that facilitate dye decolorization by *P. sanguineus* laccases, such as syringaldehyde and acetosyringone pave the path for environmentally acceptable treatments (Camarero *et al.*, 2005; Lomascolo *et al.*, 2011). Alginate composite beads containing magnetic iron oxide and activated *P. sanguineus* effectively removed malachite green from synthetic wastewater. Immobilized cells of this fungus exhibited significant absorption capacity across various pH levels, indicating suitability for acidic conditions and offering a promising approach for efficient dye removal in water remediation processes (Yang *et al.*, 2014).

Self-immobilizing fungal biomass of *P. sanguineus* was reported to decolourize Trypan Blue, an azo dye, which was directly proportional to the initial dye concentration and the reaction temperature. The decolorization process was endothermic (Annuar *et al.*, 2009). High percentage of decolorization has been noted in the case of laccases of *P. sanguineus* and manganese peroxidase when cultured on different solid and liquid substrate. These enzymes exhibited potential to degrade and decolorize synthetic dyes.

Incubation period under different culture conditions may further improve the decolorization of different dye. Reactive blue and Orange G dyes were demonstrated with 81% and 97% decolorization after 15 d of incubation which showed that the fungus played significant role in the degradation process (Christiane *et al.,* 2013). Newly isolated lacasse isoforms (Lac-I and Lac-II) from *P. sanguineus* demonstrated ability to degrade Remazol Brilliant Blue R (RBBR) dye with degradation efficiencies ranging from 82 to 88% after 3 h of incubation (Salazar-López *et al*., 2017). The polyporoid fungus was also shown to grow on King medium with Black V dye (0.05% m/v) and to possess an effective decolorization capacity under different culture conditions. It also exhibited distinct behaviour depending on the pH, brightness, and presence or absence of agitation. This fungus can also be efficiently used in the treatment of textile dye (Da Paz *et al.,* 2012).

Role in sustainable wastewater treatment

Pharmaceutical wastewaters are a group that, in terms of environmental concerns, has received little attention for a long time (Roberts and Thomas, 2006). *Pycnoporus sanguineus* has been reported to remediate three pharmaceutical wastewater samples. During the course of the treatment, it increased the synthesis of enzymes including laccase and manganese peroxidase. The mouse bone marrow micronucleus assay was performed to determine the *in vivo* genotoxic effects of treated and raw effluent, and it revealed that Mn frequency decreased in a dose-dependent way (with treated effluent). Reduced phenolics content was further directly correlated with increased laccase synthesis (Watanabe *et al.,* 2012).

Lignocellulose degradation: Unlocking the role of *Pycnoporus sanguineus* in management of agricultural waste

Pycnoporus sanguineus is one of the organisms, that plays a promising role in degrading various lignocellulosic material and might be used as their bioremediation agent. Lignocellulose, the main component of biomass which makes up around half of the plant matter created during photosynthesis and is the most prevalent renewable organic resource in soil (Seidl and Goulart, 2020). Cellulose, hemicellulose and lignin, three types of polymers are present in lignocellulosic material (Pérez *et al.,* 2002). Mycelia of *P. sanguineus* also have ability to degrade lignocellulosic material due to their highly efficient enzymatic system. Only a small fraction of the cellulose, hemicellulose, and lignin produced a by- product in forestry or agriculture is being utilized. The secretome of *Pycnoporus sanguineus* is more efficient in degrading lignocellulosic biomass than *Ganoderma resinaceum*, and that the inductive medium (*Panicum prionitis* leaves) is a promising tool for increasing glucose release levels (Gauna *et al.,* 2021). Large-scale lignocellulosic material accumulation is quite problematic and results in environmental degradation as well as the loss of potentially valuable material that can be used, among other things, to make paper, biomass fuel, compost, and also for human and animal feed. Numerous micro and macro-organism are capable of degrading and utilizing this waste as carbon source (Sánchez, 2009).

Heavy metal removal: Tackling toxic pollutant

Heavy metals such as copper, lead and cadmium are the most toxic pollutants that are introduced into natural water and have negative effects on the environment. Heavy metal pollution poses major risk to human health since these can accumulate through food chain. It has been reported that *P. sanguineus* has the ability to eliminate heavy metal in fixed-bed column studies. The experiments were designed based on the external mass transfer model and metals' diffusivity on macro fungi bed, which produced promising theoretical and experimental breakthrough profiles. Aqueous solutions containing heavy metals such as copper, lead, and cadmium can be economically removed using biosorbent by this procedure. Study on the removal of copper ions in aqueous solution was conducted using a single-metal system comprising copper ions and *Pycnoporus sanguineus* biomass to investigate the sorption capacity and effects of environmental parameters such as pH, biomass loading, temperature, and contact time. Results showed that non-living biomass of *P. sanguineus* was able to adsorb copper ions effectively (Mashitah *et al.,* 1999a). In another study, the influence of pH, initial metal ions concentration, biomass loading, and temperature on the biosorption capacity of immobilized *Pycnoporus sanguineus* cells were determined unveiled that optimum uptake of Cu (II) ions was at pH 5 with a value of 2.76mg/g. The FTIR analysis showed that OH, NH, CH, CO, COOH, and CN groups were involved in the biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus* (Yahaya *et al.,* 2009).

Pycnoporus sanguineus also plays a role in biosorption in Lead ion. Modification observed in *P. sanguineus* functional group such as lipid, amino and carboxylic in the cell through SEM and EDAX analysis, due to adsorption the of Lead ions onto the cell wall. Lead ions replaced the calcium ion present in the cell wall, indicated its role in biosorption of metal ions (Mashitah *et al.,*1999b). In a batch system including equilibrium and kinetics studies, effect of pH, initial cadmium (II) concentration, biomass loading, and temperature, biosorption of cadmium (II) ions from aqueous solution onto immobilized cells of *Pycnoporus sanguineus* was investigated The results showed that the biosorption of cadmium (II) ions increased with the increase of initial cadmium (II) concentration, pH, and temperature, also, the biosorption process is spontaneous and endothermic (Mashitah *et al.,* 2008).

As enzyme producer

A large number of foreign contaminant molecules are produced and incorporated into ecosystems as a result of the global increase in urbanization and industrial activities. Majority of these contaminants are toxic in nature and have negative influence on both human and animal health. Strategies have been used to remove these toxins from water bodies under anthropogenic stress using physical, chemical, and biological methods. Laccases, which are broad spectrum biocatalysts, have been utilized to degrade many chemicals, such as those that can be found in the effluents from businesses and hospitals, through biotechnological processes combining microbes and enzymes (Dong *et al.*, 2023). “Mycofiltration” is an environmentally friendly technology utilizing fungal mycelia to treat contaminated water. The potential use of this technology is to filter out and reduce levels of organic, inorganic and microbial contaminants in wastewater or effluent. This technique is cost-effective and natural process with no hazardous or toxic end product (Mnkandla and Otomo, 2021). When employing free enzymes or crude enzymes, laccases have demonstrated significant potential in the biotransformation of a variety of contaminants. However, large-scale production of laccases is required to solve this problem. *Pycnoporus sanguineus* turns out to be a promising candidate for the solution to the problem of laccase production issues because it generates a high redox potential laccase that is simple to scale up for industrial purposes. Several studies have been carried out to enhance the production of laccase by this species, which could be used in bioremediation (Arregui *et al.,* 2019).

In another study, 30 strains of *Pycnoporus sanguineus* and *P. coccineus* collected from subtropical and tropical environment of different geographical locations i.e. French Guiana (South America), French New Caledonia (Pacific Islands), China, Vietnam, Australia, and Venezuela were deposited in the International Centre of Microbial Resources-Banque de Ressources Fongiques de Marseille collection (CIRM-BRFM, INRA, Marseille, France). Out of these 30 strains, 3 strains, BRFM 938, BRFM 66, and BRFM 902, were selected for purification and characterization after primary screening. The study revealed *P. sanguineus* and *P. coccineus* as exceptional producers of high redox potential laccases that are simple to scale up for industrial production and were considered ideal for white biotechnology techniques due to their highest thermo (75–78℃) and pH (5–7) stability. BRFM 66 laccase had the ability to oxidize most nonphenolic compounds and decolorized dye (Uzan *et al.*, 2010)**.** Synthesis of laccase by solid state fermentation (SSF) using agricultural residue such as Sago (hampas), oil palm frond parenchyma tissue (OPFPt), rubberwood and sawdust by altering the inoculum's age, density, and nitrogen supplementation. The results indicated that OPFPT and Sago based solid state fermentation produced the maximum laccase for *P. sanguineus*. Further, the extraction and productivity of laccase was maximum with use of tap water at pH-5 (Vikineswary *et al.*, 2006)**.** *P. sanguineus* and its laccase isolated from oil-polluted site both demonstrated significant thermostability. This makes them suitable for biotechnological applications like bioremediation (Gonzalez *et al*., 2008).

Another study revealed that laccase from *P. sanguineus* showed maximum thermal stability when recombinate with *Trichoderma reesei* and could effectively break down the hazardous phenolic compound bisphenol A. The study reported that, it has considerable potential to cleanse household trash and wastewater that contained bisphenol A (Zhao *et al.,* 2018). Different carbon and nitrogen sources (maltose, glucose, fructose and sucrose as carbon source; ammonium tartrate, sodium nitrate, asparagine and yeast extract as nitrogen source) and nitrogen concentration significantly affected the activity of lacasse. Sucrose and asparagine-based medium provided the maximum lacasse activity (Eugenio *et al*., 2009). *Pycnoporus sanguineus* can also be utilized for phenolic degradation, pulp bleaching, and dye decolorization, as these processes withstand the maximum temperature (60°C). *P. sanguineus* is able to endure this temperature. Enzyme laccase production was studied by using bark shaving as substrate under semi-solid-state-fermentation through Response Surface Method (RSM). Under optimal condition and appropriate culture medium the production level of laccase enzyme increased significantly. Maximum laccase activity of roughly 16000 UL^{-1} was attained when supplemented with Cu^{2+} which also showed a great potential for decolorization of the dye namely Reactive Black 5 (RB5), Acid Red 88 (AR88) and Lanaset Grey G (LG) This enzyme can be used to treat wastewater containing dyes (Gioia *et al*., 2014).

P. sanguineus CBS614.73, a strain, was reported to generate a different phenoloxidase, a novel monomeric tyrosinase with a specific activity of 30 and 84 U mg^{-1} protein for monophenolase and diphenolase, respectively. This protein could be used as a source of synthesis of antioxidant compounds and used in the cross-linking of proteins (Halaouli *et al.,* 2005). Hydrophobic interaction chromatography with syringaldazine used as a substrate to purify laccase from *P. sanguineus* CCT-4518. At 50°C for 24 and 48 h, respectively, this laccase was highly thermostable and commercially important (Garcia *et al*., 2007). *P. sanguineus* CCT-4518 was investigated for tyrosinase production using L-tyrosine as an inducer. It was indicated that this macrofungus produces thermal stable enzyme for industrial interest under optimized conditions which was confirmed by non-denaturing polyacrylamide gel electrophoresis (Duarte *et al.,* 2012).

CONCLUSION

Pycnoporus sanguineus exhibits great potential for advancing environmental biotechnology, particularly in wastewater treatment and dye decolorization. Future research should focus on optimizing enzyme production and activity to enhance the efficiency of dye decolorization and pollutant degradation. Additionally, large-scale applications should investigate cost-effective methods for cultivating *P. sanguineus* and utilizing lignocellulosic waste as substrates, further promoting sustainability. Overall, leveraging the capabilities of *P. sanguineus* can contribute significantly to environmental remediation, offering an eco-friendly alternative in industrial processes and supporting global sustainability goals.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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