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Utilization of *Leiotrametes menziesii* BRB 73 for Decolorization of Commercial Direct Dyes Mixture with Different Culture Conditions

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ABSTRACT

Mycoremediation is classified as an inexpensive, environmentally friendly, and effective technique to reduce wastewater. *Leiotrametes menziesii* BRB 73 was one of the White Rot Fungi (WRF) that has the potential to degrade dyes. Suitable environmental conditions can optimize dye decolorization results. This study aims to investigate optimal environmental conditions such as time incubation, concentration of dyes, pH, $CuSO_4$, and glucose concentration against decolorization of a mixture of direct dyes and enzyme activity (laccase and MnP). The mixture of commercial direct dyes used contains direct turquoise (DT), direct orange (DO), and direct yellow (DY) dyes. Decolorization was measured using a spectrophotometer at 400-700 nm. Laccase and MnP assay using ABTS and 2.6 DMP as substrate, respectively. The highest decolorization by *Leiotrametes menziesii* BRB 73 was produced at 54.3% at 96 hours and increased to 67% at a dye concentration of 500 $mg.L^{-1}$. Meanwhile, the highest laccase and MnP activities were 215 $U.L^{-1}$ and 39 $U.L^{-1}$, respectively. pH range was quite wide, ranging from pH 5.5-9, supported by stable MnP activity from pH 3-7. $CuSO_4$ inducers were not required for the decolorization of these dyes. Decolorization was optimal at the addition of 1% glucose, while enzyme activities were 0.5% glucose. Decolorization of dyes by *Leiotrametes menziesii* BRB 73 was indicated through degradation pathways involving laccase and MnP enzymes. This isolate has a high tolerance to dye concentrations, a wide pH range, and low carbon requirements. Thus, it was recommended as a mycoremediation agent.

INTRODUCTION

Synthetic dyes are widely used in the textile industry, with more than 10,000 different dyes and pigments (Singh et al. 2023). The release of dyes into the environment causes a negative impact on water quality because it lowers the dissolved oxygen concentration and makes it difficult for light to penetrate the water, thus adversely affecting aquatic organisms (Ali et al. 2022). In addition, it can cause toxicity, carcinogenetic, and mutagenetic (Asgher et al. 2020).

Many chemicals (Fenton's reagent, ozonation, oxidation process, etc.) and physical methods (coagulation, adsorption, membrane filtration, flocculation, etc.) for removing dyes are available (Monga et al. 2022, Sudhaparimala & Usha 2022, Vinotha & Leema Rose 2023). However, these methods have their disadvantages which are: selective over types of dyes, low efficiency, sludge production, sometimes high cost, and generation of toxic by-products (Monga et al. 2022). Biodegradation by fungi is a relatively environmentally friendly, cost-effective, and effective method (Akhtar & Mannan 2020). Recently, White Rot Fungi (WRF) has

become a concern in handling dyes in the environment. This fungus has promising extracellular enzymes for degradation due to its wide substrate specificity and mild catalysis conditions such as laccase and manganese peroxidase (Dao et al. 2021, Ridtibud et al. 2024) (Table 1). The enzymes have good prospects in the degradation of dyes generated from textile industry waste (Kumar & Chandra 2020). WRF that produces these enzymes promises to degrade dyes effectively (Zafar et al. 2022), such as *Rigidoporus* sp. FMD21(Dao et al. 2021), *Coriolus versicolor, Pleurotus ostreatus* (Afiya et al. 2019), and *Trametes polyzona* (Pérez-Cadena et al. 2020), *Leiotrametes menziesii* (BRB 73) (Apriani et al. 2024).

Laccase and MnP are ligninolytic enzymes which are classified into two, namely phenol oxidase (laccase), and heme peroxidase (MnP) (Suryadi et al. 2022). Laccase (benzenediol: oxygen oxidoreductase; EC1.10.3.2) is a group of copper-containing polyphenol oxidases, which utilize molecular oxygen as the final electron acceptor (Bittencourt et al. 2023). Laccases can mediate coupling reactions that form the basis for dye removal (Kyomuhimbo & Brink 2023). This enzyme is capable of oxidizing various substrates such as ortho and para diphenols, phenolic acids, aromatic amines, methoxy-substituted phenols, and several other compounds (Herath et al. 2024). Thus, it makes laccase able to decolorize synthetic dyes of a wide spectrum of dyes such as RBBR, acid orange, direct green, direct blue, reactive red, malachite etc. (Kyomuhimbo & Brink 2023).

Manganese peroxidase (MnP, or Mn (II): H_2O_2 oxidoreductase, EC 1.11.1.13) is an extracellular glycoprotein containing heme as a prosthetic group (Suryadi et al. 2022). It catalyzes the oxidation of Mn^{2+} to Mn^{3+} in a multistep process (Herath et al. 2024). Furthermore, Mn^{3+} acts as a mediator in the process of oxidation for some phenolic and non-phenolic compounds (Kumar & Chandra 2020). It can catalyze the oxidation of a large spectrum of phenolic compounds, including toxic dye pollutants (Bilal et al. 2019).

Both laccase and MnP produced by WRF play a major role in the degradation of synthetic dyes (Bankole et al. 2018). However, fungal growth, extracellular enzyme production, and dye decolorization are determined by environmental factors such as pH, mediators, dyes, and carbon concentrations (Rajhans et al. 2021). Each WRF has different optimal environmental conditions, so this study needs to be carried out to determine the optimal conditions of *Leiotrametes menziesii* BRB 73 in the decolorization of mixtures of commercial direct dyes. In this study, we investigated the effect of various environmental conditions, such as time incubation, concentration of dyes, pH, $CuSO_4$, and glucose concentration for decolorization of mixture direct dyes by BRB 73. In addition, the relationship between environmental condition and enzymatic activities (Laccase and MnP).

MATERIALS AND METHODS

Culture Condition and Maintenance

Leiotrametes menziesii BRB 73 obtained from the culture collection of the Research Center for Applied Microbiology, BRIN was maintained and grown on a solid medium of malt extract agar (MEA). Isolate was cultivated on MEA at 25±3°C for 7 days before treatment. Three plugs of isolate were grown in MGP broth containing, malt (20 $g.L^{-1}$), glucose (20 $g.L^{-1}$), and peptone (1 $g.L^{-1}$) at 25±3°C (room temperature) for 7 days. A corkborer (diameter 0.5 cm) was used to form the plug. Cultures were added a mixture of commercial direct dyes (direct turquoise=DT, direct orange=DO, and direct yellow=DY) up to a concentration of 100 $mg.L^{-1}$. The supernatant of fungal cultures was centrifuged at 10.000 rpm at room temperature for 10 minutes within 96 after incubation for decolorization assay (Alam et

Table 1: Decolorization of direct dyes by White Rot Fungi.

WRF	Dyes	% decolorization	Enzyme	Reference
Trametes versicolor	Direct Green 6 Direct Blue 15 Direct Orange 26 Direct black 38 Direct Yellow 12	DG6: 76% DB15: 98% DO26: 92% DB38: 85% DY12: 70% (50 $mg.L^{-1}$ of dyes within 3 days of incubation)	Laccase	Pazarlioglu et al. 2010
Pleurotus flabellatus	Direct Blue 14 (DB14)	90.39% decolorization of 20 mg/L dyes for 6 hours incubation	Crude enzyme (MnP and Laccase)	Singh et al. 2013
Coriolopsis sp. Strain arf5	Direct Blue 71 (C.I. 34140)	40 to 90% of 200 $mg.L^{-1}$ DB71 within 5 days	Laccase	Cheng et al. 2016
Trametes hirsuta EDN084	Direct Blue 71	85%	Laccase	Chaurasia & Bharati 2019
Ganoderma lucidum EN2	Direct Red 5B (DR5B)	40.34% to 95.16% of 175 $mg.L^{-1}$ DR5B within 48 h		Sun et al. 2023)

al. 2021). Meanwhile, the supernatant was directly used for enzyme activities assay.

Optimation of Culture Conditions

To investigate cultural conditions, the experiment cultured isolates for 96 hours with different dye concentrations (100, 200, 300, 400 and 500 mg.L^{-1}) (Omar 2016). pH effect was monitored in the acidic to alkaline range, including pH 3, 3.5, 4, 4.5, 6, 6.5, 7, 7.5, 8, 8.5, and 9. Culture conditions were regulated using NaCl and NaOH (Colla et al. 2020). Copper sulfate ($CuSO_4$) (0, 1, 2, 3, 4, 5 mM) was added to the medium to determine their impact on decolorization and enzyme activities (Afreen et al. 2018, Zhu et al. 2016). Concentrations of glucose added in culture were 0, 0.5%, 1%, 2%, 3%, 4%, 5% and 10% (Alhomaidi et al. 2023). All experimental units were repeated 3 times.

Decolorization Assay

Percent decolorization was calculated after 96 hours of incubation using a TECAN infinite 200 pro microplate reader (Switzerland) (interval 400-700 nm). It was calculated according to formula: D = 100 [(A0-A1)/A0], where D is decolorization (%). A0 is initial absorbance. A1 is absorbance after decolorization (Anita et al. 2021).

Laccase Activity Assay

Laccase activity was measured using a TECAN infinite 200 pro microplate reader (Switzerland) at 420 nm for 60 s. ABTS was used as a substrate at room temperature. The assay mixture contained 250 µL of ABTS 2 mM, 50 µL of culture filtrate, and 200 µL of acetate buffer 0,1 M (pH.4.5). Laccase activity (U mL^{-1}) with molar absorptivity ($\mathcal{E}$) of 36,000 M^{-1} cm^{-1} was calculated according to formula Alam et al. (2021):

$$\text{Laccase activity } (\text{U.mL}^{-1}) = \frac{(Abs.(t) - Abs(0)\ x\ Vtotal\ mixture\ (mL)\ x\ 10^3}{\mathcal{E}xV.enzyme\ (ml)\ x\ t\ x\ d} \quad \ldots(1)$$

Abs. (0) = the initial absorbance Abs. (t) = the final absorbance

10^3 = correction factor (µmol/mol) d = length of the cell

t = reaction time (1 min)

Manganese Peroxidase Activity Assay

Manganese peroxidase (MnP) activity was measured using a TECAN infinite 200 pro microplate reader (Switzerland) at 470 nm for 60 s. The assay mixture contained 100 µL culture filtrate, 12.5 µL 2,6 DMP 20 mM, 175 µL malonic buffer 50 mM (pH 4.5), 12.5 µL $MnSO_4$ 20 mM, 30 µL H_2O_2 2 mM. MnP activity (U mL^{-1}) was calculated according to the formula (Anita et al. 2021):

$$\text{MnP activity } (\text{U.mL}^{-1}) = \frac{(Abs.(t) - Abs(0) \times Vtotal\ mixture\ (mL) \times 10^3}{\mathcal{E} \times V.enzyme\ (ml) \times t \times d} \quad \ldots(2)$$

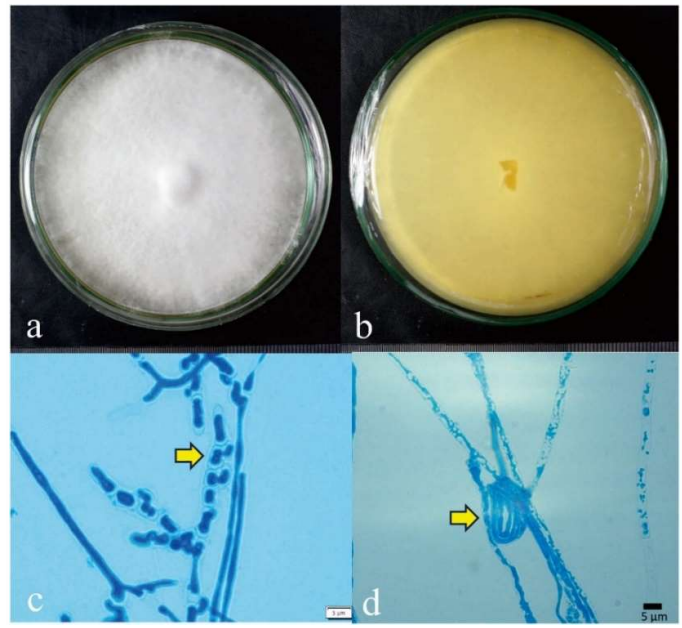

Fig. 1: Microscopic feature characteristic of *Leiotrametes menziesiii* BRB 73 after 5 days post inoculation; a) Mycelium of *L. menziesii* BRB 73 showed to be white, and flattened, (b) with a yellowish color mycelium on the reverse side, (c) The hyphae were thick-walled, hyaline, with clamp connection which width of the hypha varied from 1.77-3.56 µm, (d) and coil hyphae.

Abs. (0) = the initial absorbance Abs. (t) = the final absorbance

10^3 = correction factor (μmol/mol) (Ɛ) = 46,600 M^{-1} cm^{-1}

t = reaction time (1 min) d = length of the cell

RESULTS AND DISCUSSION

Leiotrametes menziesii BRB 73 was a potential White Rot Fungus (WRF) that has not been widely reported for the decolorization of a mixture of direct dyes. *Leiotrametes menziesii* can be found in various host plants including *Acacia* sp, *Arthocarpus altilis, Cocos nucifera, Horsfieldia* sp., *Mangifera indica, Morinda citrifolia, Rhizopora* sp., and other plants (Park et al. 2021). This fungus has white hyphae, a degree of growth rate of 11.71 mm/day within 5-7 days on MEA media at 27± 2°C (Fig. 1). It produces lignocellulolytic enzymes consisting of laccase and MnP (Alexandropoulou et al. 2017). Moreover, different fungi have different environmental conditions in the decolorization of dyes. The data obtained showed that environmental conditions have an impact on the ability of fungi to remove dyes.

Time Incubation

Incubation time determines the rate of fungi to decolorize the dyes shown in Fig. 2 and Table 2. The highest decolorization by BRB 73 occurred at the final observations, which showed that this fungus had not yet reached the stationary phase. So, it was indicated that optimal incubation had not been obtained. This occurs in *Aspergillus niger* where decolorization of various synthetic dyes occurs after 96 hours (4 days) of incubation (Omar 2016).However, other cases showed that decolorization of Congo red by *Aspergillus quadrilineatus* occurred after 72 hours of incubation (Yusuf et al. 2023).

Table 2: Effect time on decolorization of 200 mg.L^{-1} mixture of commercial direct dyes and enzyme production.

Time	Decolorization (%)	Laccase activity (U.mL^{-1})	MnP activity (U.mL^{-1})
24	6.2 ± 0.3	0.0 ± 0.0	0.8 ± 0.2
48	13.8 ± 0.9	50.4 ± 2.3	7.4 ± 1.3
72	23.3 ± 2.8	123.4 ± 4.8	20.5 ± 0.5
96	54.3 ± 0.6	164.4 ± 2.5	30.2 ± 4.0

Increased decolorization occurs until final incubation (96 H), which was followed by increased enzyme activity (laccase and MnP) (Table 2). It was proved that laccase and MnP play a major role in dye decolorization. Fungi produce enzymes that have similar abilities such as *P. prosopidis* (Bankole et al. 2018). Some White Rot Fungi degrade dyes into simple products by involving ligninolytic extracellular enzymes such as laccase and MnP to mineralize the dyes (Chaturvedi 2019). The mechanism involves the reaction of hydroxylation, demethylation, and cleavage ring (Shindhal et al. 2021).

Effects Of Environment Conditions on The Percentage of Decolorization and Biomass by BRB 73

Dyes concentration in this study ranged from 100-500 mg.L^{-1}. Fig. 3 shows that a high dye concentration results in increased dye removal, which ranges from 53-67%. Biomass has increased, although not significantly ranging from 0.12-0.13 g. Thus, it was found that BRB 73 can tolerate the highest concentration of commercial direct dyes. The dye concentration of 500 mg.L^{-1} showed no

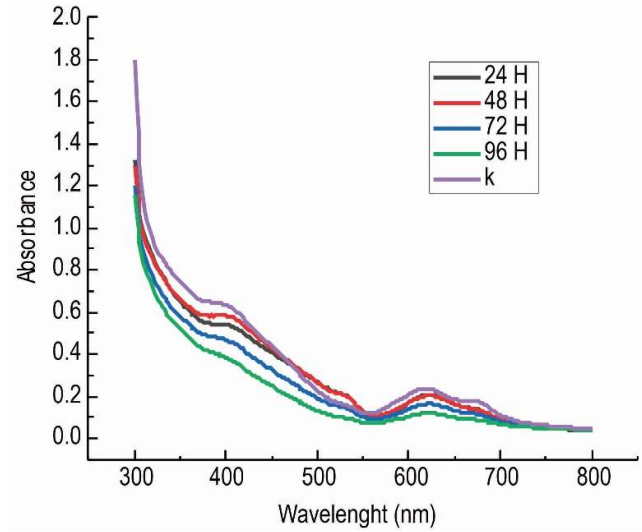

Fig. 2: Decolorization of commercial mixture dyes (200 mg.L^{-1}) by BRB 73 at various incubation times.

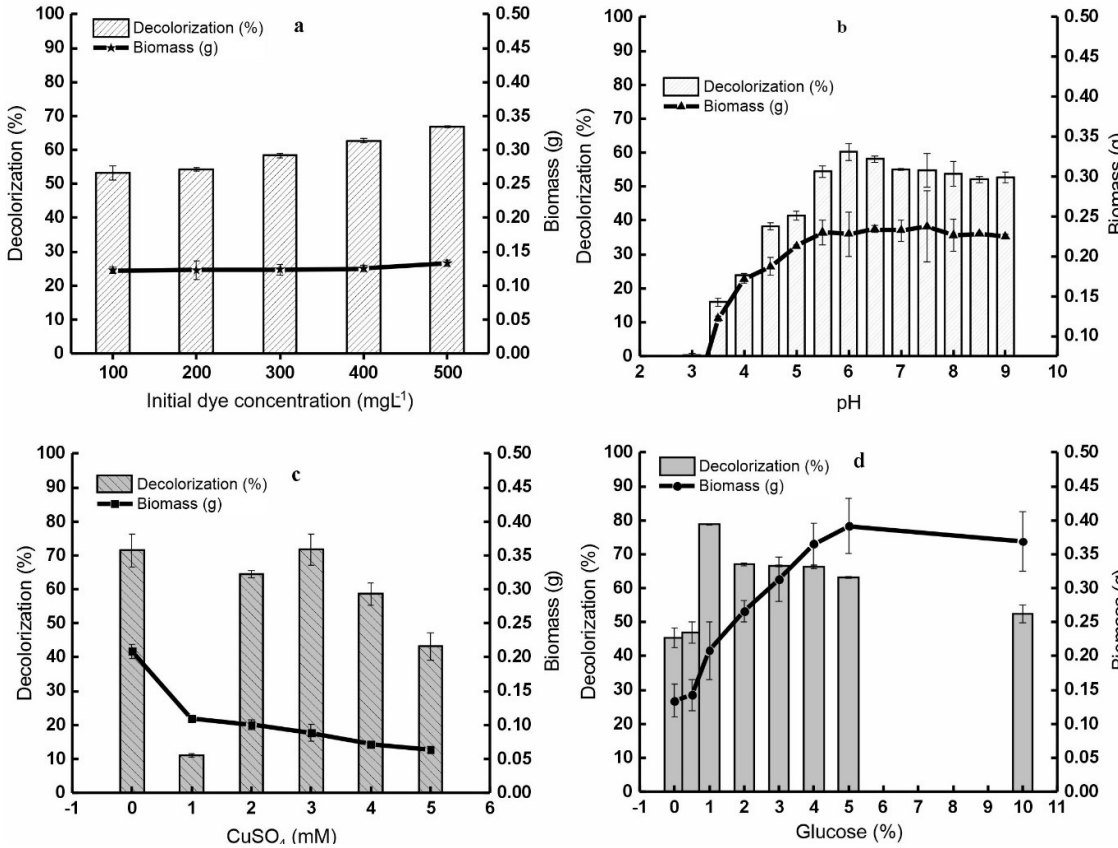

Fig. 3: Impact of environmental condition on dye decolorization and biomass by *Leiotrametes menziesii* BRB 73. dye concentration (a), pH (b), $CuSO_4$ (c), Glucose (d).

toxic symptoms, which was supported by stable biomass (Fig. 3a). Thus, it was indicated that BRB 73 was effective in commercial dye removal because it has a high tolerance to commercial direct dyes. In contrast to other studies, which showed that increased concentration leads to a decrease in the percentage of decolorization, such as *Ceriporia lacerate* (Wang et al. 2017), *Coriolus versicolor* and *Pleurotus ostreatus* (Afiya et al. 2019). The increased number of dye molecules causes fungal stress that inhibits fungal growth because the amount of enzymes produced to degrade dyes decreases (He et al. 2018).

Fungal growth is influenced by pH, generally, fungi grow at low pH which ranges from pH 4-5 (Sen et al. 2016). Each fungal species has a different favorable pH range, as shown in a study of seven *Lentinus* strains by Kalaw et al. (2021). Fig. 3B shows that mycelium growth increases from pH 3 to pH 5.5 Then, it becomes stable from pH 5.5 to pH 9. It can be indicated that this fungus has growth in a wide pH range. Other fungi that have a wide pH range are *G. lucidum* (Subedi et al. 2021), and *Lentinus swartzii* (Dulay et al. 2021). The graphic profiles of the percentage of decolorization and biomass were similar. However, the highest decolorization at pH 6 was 60%. The optimum fungus at pH 6 in the absorption of Remazol Red RB Textile dyes was *Saccharomyces cerevisiae* (Sukarta et al. 2021). Decolorization decreases although not significantly when the alkaline value of the solution increases at pH 9 to 52%. In contrast to the research of Yusuf et al. (2023),the decrease in the percentage of congo red decolorization was drastic by *Aspergillus quadrilineatus*, with optimum decolorization at pH 5 was above 70% but drops dramatically at pH 7 to below 50%.

Inducers in dye decolorization were widely studied, including the copper sulfate group known to increase the production of laccase enzymes in WRF (Jaramillo et al. 2017) and increase dye degradation (Jiménez et al. 2019). Fig. 3C shows the impact of $CuSO_4$ inducers on fungal growth and dye decolorization. Increased inducer concentration resulted in stunted growth of BRB 73, which was indicated by decreased biomass, from 0.2 g to 0.06 g. It showed that high concentrations of $CuSO_4$ were toxic to fungi. According to Jiménez et al. (2019),the toxicity of heavy metals to microorganisms depends on concentration. It was

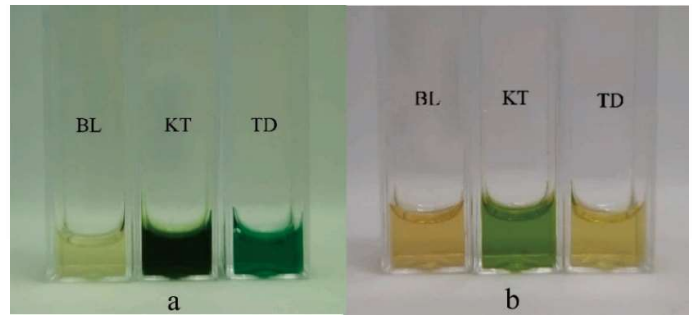

Fig. 4: Decolorization mixture of commercial direct dyes by BRB 73; a) 500 mg.L^{-1}; b) 100 mg.L^{-1} mixture dyes with added 1% glucose (optimum condition); BL (MGP medium), KT (before treatment), TD (after treatment).

observed that $CuSO_4$ concentrations between 0.1 and 1 mM did not inhibit growth. However, $CuSO_4$ concentrations higher than 1 mmol/L inhibit the growth of *P. ostreatus* mycelium (ACCC 52857) (Zhu et al. 2016). Meanwhile, the decolorization curve shows an unequal profile. The percentage of decolorization, both untreated and treated (3 mM), was 71.4% and 71.8%, respectively. It was indicated that $CuSO_4$ was not required as an inducer of BRB 73 in liquid cultures for the decolorization of these commercial dyes.

Glucose was utilized WRF in culture as a carbon source, evident from an increase in biomass from 0.132 g to 0.39 g (Fig. 3D). However, the addition of 10% of glucose resulted in a decrease in biomass to 0.37 g. Dye decolorization was optimal at a glucose concentration of 1%, which was 79%. Rajhans et al. (2021) recommend 1% glucose as the optimum concentration in the decolorization of azo dyes (Fig. 4). The decolorization profile curve showed a decrease while the glucose concentration increased. Similar study results occur in *Ganoderma multistipitatum*, increased glucose concentration can decrease dye decolorization (Alhomaidi et al. 2023). Due to high glucose concentrations, WRF mycelium prefers glucose degradation rather than complex molecular dyes (Senthilkumar et al. 2014). Fungi chose a simple carbon source that was utilized first (Hamad et al. 2014).

The Effect of Environmental Conditions on Laccase Activity

In this study, laccase plays a role in dye degradation, as seen from the increase in the activity of this enzyme according to the increase in the percentage of decolorization (Fig. 5A). Laccase increased from 154 $U.L^{-1}$ to 215 $U.L^{-1}$ indicates the presence of high dye concentrations capable of encouraging the production of laccase enzymes, as occurs in *T. versicolor* (Vel et al. 2020). Laccases are extracellular oxidoreductase enzymes reported by Bittencourt et al. (2023) to play a role in the decolorization of synthetic dyes. According to Castillo et al. 2023), laccases produced by fungi are more attractive for bioremediation applications because these enzymes are not specific to the substrate and have a high potential for oxidation of a diverse range of pollutants, such as synthetic dyes. Degradation by enzymes produced by fungi is better than bacteria or algae (Sen et al. 2023). Laccase activity has not reached the highest values indicated by an increase in the percentage of decolorization. According to Vel et al. (2020) enzymes that reach the highest values show decolorization in the exponential or early stationary phase.

pH has a significant impact on the levels of laccase enzyme produced. The difference in contrast in laccase activity between Fig. 5A and 5B, shows that the addition of NaOH and HCl has an impact on decreasing laccase activity. It suggests that laccase was intolerant to the compound. According to Coria-Oriundo et al. (2021) laccase that was not stable in a wide pH range, intolerant to organic compounds, and high salts cannot be recommended on an industry scale. The highest laccase activity at pH 6 was 13 $U.L^{-1}$ (Fig. 5B). Suryadi et al. (2022) reported that excessive laccase production by fungi was between pH 4.5 and 6. *O. canarii* shows maximal laccase activity at pH 4.5–5.5 (Lark et al. 2019).

$CuSO_4$ was presented in the culture medium as a mediator to increase laccase production by inducing laccase gene transcription (Dur et al. 2022). However, the presence of $CuSO_4$ in the culture medium decreases laccase activity by BRB 73, so it was indicated that the presence of $CuSO_4$ did not contribute to laccase production (Fig. 5C). It happens because the type of metal inducer and the concentration used are indicated not yet suitable for this WRF, similar facts occur in *Aspergillus flavus* with a concentration of 5 mM-10 mM (Liu et al. 2020). Laccase production by *Pleurotus ostreatus* MTCC 142 can be increased by the addition of Cu^{2+} (Das et al. 2016), while mediators for *M. arundinis* were HBA and HBT (Mendes et al. 2022). The optimum concentration of $CuSO_4$ for *A.maxima* was 0.1-3.0 mM (Kumar & Chandra 2020), while for *T. Versicolor* was 0.1 and 1 mM (Jaramillo et al. 2017).

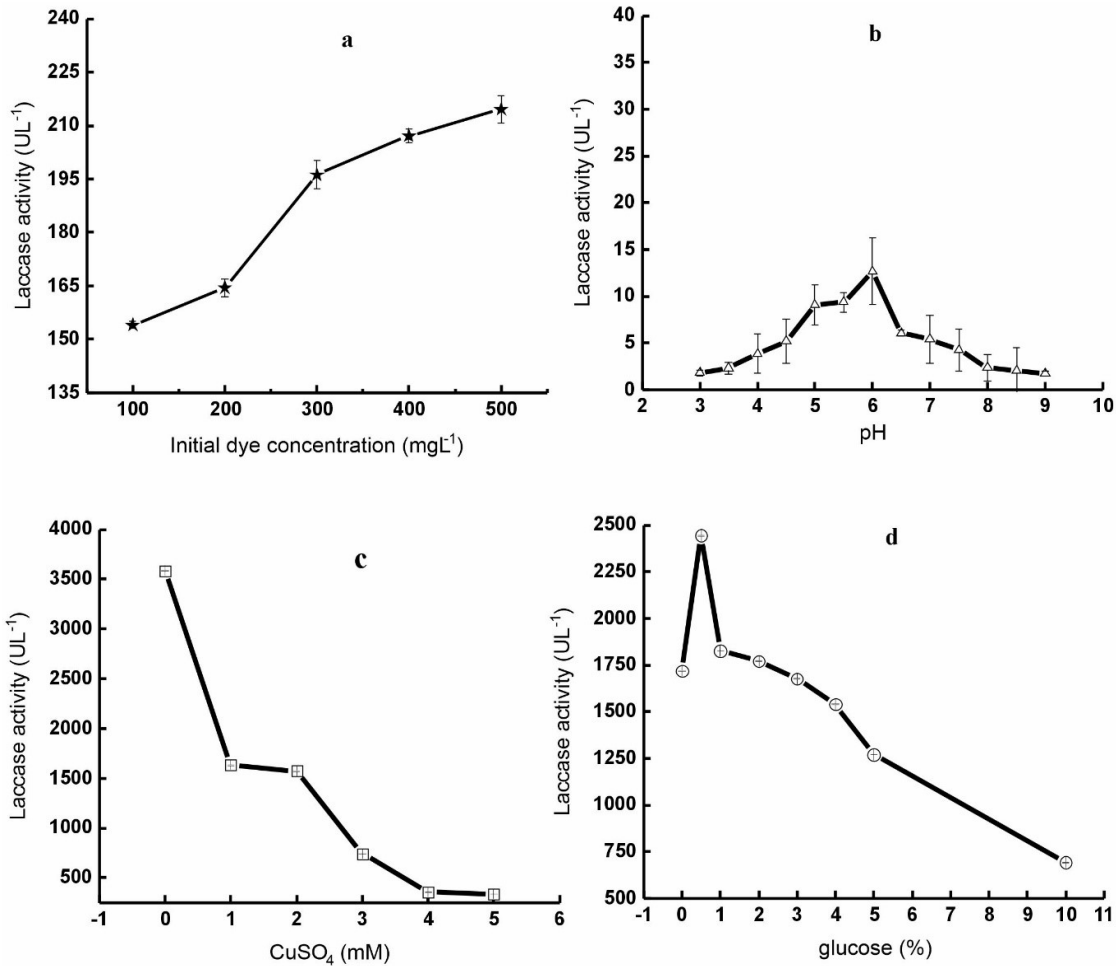

Fig. 5: Impact of environmental conditions on laccase activity by *Leiotrametes menziesii* BRB 73; (a) dye concentration, (b) pH, (c) $CuSO_4$, (d) glucose.

The curve profile between Figs. 5D and 2D have similarities. However, a glucose concentration of 0.5% stimulated the highest laccase production, which was 2444 $U.L^{-1}$. It was verified that a low concentration of glucose stimulates high laccase production (Daniel et al. 2018). The lowest laccase activity was at 10% glucose addition, which was 691 $U.L^{-1}$. High glucose concentrations result in both decreased laccase activity and decolorization. Suppression of laccase synthesis due to high concentrations also occurs in *Trametes pubescens* (Alhomaidi et al. 2023).

In this study, the nutrients (nitrogen and glucose) of BRB 73 were insufficient so $CuSO_4$ added to the medium did not affect laccase activity. According to Duran-Sequeda et al. (2022), copper was involved in the induction of laccase if the conditions of the nutrient source (nitrogen and glucose) were sufficient. This nutrient affects the regulation of the laccase gene. In *P. ostreatus,* this condition can express transporter genes (CTRs) to uptake copper from the environment, resulting in an increase in laccase activity up to 20 times.

The Effect of Environmental Conditions on MnP Activity

Enzyme MnP was produced by BRB 73 during direct commercial synthetic dye decolorization. Their activities were much lower than laccase. However, this enzyme profile also shows improvement (Figs. 6A & 6B). MnP Activities at dye concentrations of 100 $mg.L^{-1}$ and 500 $mg.L^{-1}$ were 16 $U.L^{-1}$ and 39 $U.L^{-1}$, respectively. It indicates that decolorization involves the MnP enzyme as well. According to Amaro Bittencourt et al. (2023), MnP produced by *Trametes* sp. 48424 demonstrates high dye decolorization capability. Fungi can produce more than one enzyme in the decolorization process, such as laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP) (Noman et al. 2020). All three enzymes were detected in RB5 decolorization by *P. chrysosporium* ATCC 24725

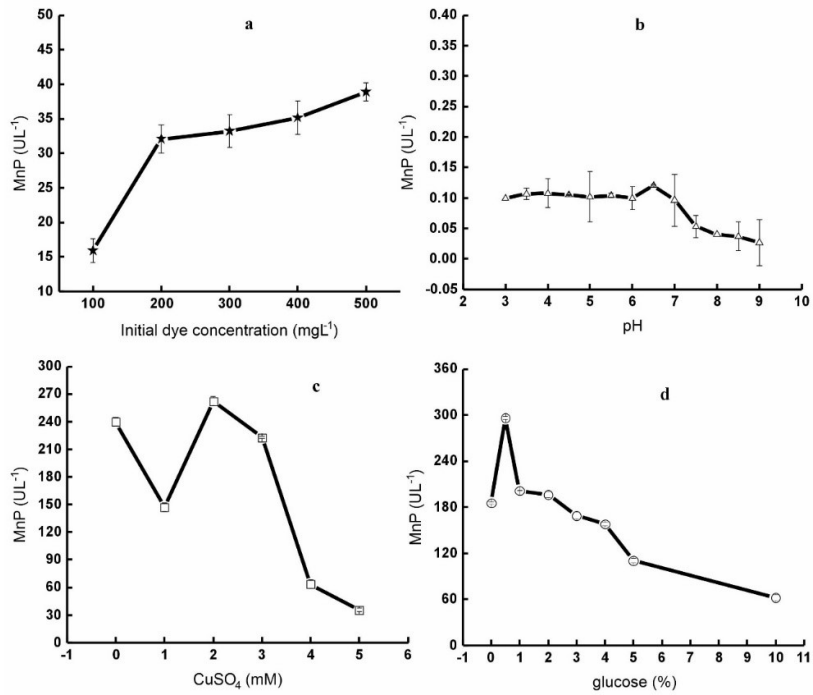

Fig. 6: Impact of environmental conditions on MnP activity by *Leiotrametes menziesii* BRB 73; (a) dye concentration, (b) pH, (c) $CuSO_4$, (d) glucose.

(Permpornsakul et al. 2016). *Trichoderma tomentosum* was indicated to involve two enzymes, namely manganese peroxidase and lignin peroxidase in decolorizing acid red 3 (He et al. 2018). The combination of various enzymes can significantly improve the biodegradation of textile dyes. However, the combination of enzymes depends on the type of dye, chemical structure, and microbes involved (Das et al. 2023).

pH affects enzyme production, as occurs in laccase production. MnP activity showed a stable value ranging from pH 3 to 7, which was 0.1 $U.L^{-1}$. Stability of MnP by *Echinodontium taxodii* 2538 at pH range 2-6 (Kong et al. 2016). The optimum pH of WRF for producing MnP was different, for example, *Clitopilus scyphoides* and *Cerrena unicolor* BBP6, which were 5.2 and 4.5, respectively (Rao et al. 2019). However, MnP decreased to 0.03 $U.L^{-1}$ at pH 9. The overall data showed that the production of both enzymes, laccase and MnP, were lower than in other treatments (Figs. 5 & 6). Nevertheless, however, it was not affected by decolorization values ranging from 50-60%.

The impact of increasing $CuSO_4$ concentration causes a decrease in MnP activity (Fig. 6C). MnP activity was highest in additions of 2 mM, which was 262 $U.L^{-1}$. Whereas, the lowest MnP activity was 35 $U.L^{-1}$ (5 mM of $CuSO_4$). Each WRF species requires a different concentration of $CuSO_4$ inducers to obtain optimum enzyme activity and decolorization (Laksmi et al. 2021).

MnP production decreases with the addition of glucose (Fig. 6D). The lowest glucose concentration (0.5%) was able to produce the highest MnP enzymes, which was 296 $U.L^{-1}$. Stress culture conditions can increase the MnP enzyme (Yuan et al. 2022). Meanwhile, the lowest activity was 62 $U.L^{-1}$ at a concentration of 10% glucose. Other research information, shows that the type of carbon source affects the activity of MnP such as *P.ostreatus* which produces higher MnP using carbon sources from xylan (Daniel et al. 2018). Other fungi such as *Trichoderma harzianum* prefer sucrose over other carbon sources (Kumar & Arora 2022). Even without the addition of glucose, the presence of MnP was detected, which was 185 $U.L^{-1}$. However, the percentage of decolorization was 45%. In contrast, *M. palmivorus* VE111 produces high MnP at high glucose concentrations (30 $g.L^{-1}$), which was 59 $U.L^{-1}$ (Daniel et al. 2018).

CONCLUSIONS

Leiotrametes menziesii BRB 73 has potential as a dye decolorization agent. It decolorized 54.3% of commercial direct dyes within 96 hours. Whereas, it was tolerance to dyes concentration exceeding 500 $mg.L^{-1}$ which was supported by stable mycelium growth, increased laccase, and MnP enzyme activity. The decolorization pH range was quite wide, ranging from pH 5.5-9, supported by stable MnP activity from pH 3-7. However, the optimal condition of the laccase enzyme

was at pH 6. $CuSO_4$ inducers were not required by BRB 73 so it was necessary to study other types of inducers. Decolorization was optimal at the addition of 1% glucose, while enzyme activity was optimal at the addition of 0.5% glucose. This WRF produces extracellular enzymes including laccase and MnP during the decolorization process under various environmental conditions, which were indicated to play a major role in dyes degradation. Thus, it was recommended as a mycoremediation agent.

REFFERENCES

Afiya, H., Ahmet, E.E. and Shah, M.M., 2019. Enzymatic decolorization of Remazol Brilliant Blue Royal (RB 19) textile dye by White Rot Fungi. *Journal of Applied and Advanced Research*, 4(1), pp.11-15.

Afreen, S., Anwer, R., Singh, R.K. and Fatma, T., 2018. Extracellular laccase production and its optimization from *Arthrospira maxima* catalyzed decolorization of synthetic dyes. *Saudi Journal of Biological Sciences*, 25, pp.1446-1453.

Akhtar, N. and Mannan, M.A., 2020. Mycoremediation: Expunging environmental pollutants. *Biotechnology Reports*, 26, p.e00452.

Alam, R., Ardiati, F.C., Solihat, N.N., Alam, M.B., Lee, S.H., Yanto, D.H.Y., Watanabe, T. and Kim, S., 2020. Biodegradation and metabolic pathway of anthraquinone dyes by *Trametes hirsuta* D7 immobilized in light expanded clay aggregate and cytotoxicity assessment. *Journal of Hazardous Materials*, 405, p.124176.

Alexandropoulou, M., Antonopoulou, G., Fragkou, E., Ntaikou, I. and Lyberatos, G., 2017. Fungal pretreatment of willow sawdust and its combination with alkaline treatment for enhancing biogas production. *Journal of Environmental Management*, 203, pp.704-713.

Alhomaidi, E.A., Umar, A., Alsharari, S.S. and Alyahya, S., 2023. Evaluation of Lacc134 oxidoreductase of *Ganoderma multistipitatum* in detoxification of dye wastewater under different nutritional conditions. *Microbiological Research*, 14, pp.1398-1412.

Ali, S.S., Umar, A., Alsharari, S.S. and Alyahya, S., 2023. Evaluation of Lacc134 oxidoreductase of *Ganoderma multistipitatum* in detoxification of dye wastewater under different nutritional conditions. *Microbiological Research*, 14, pp.1398-1412.

Anita, S.H., Ningsih, F., Heri, D. and Yanto, D.H.Y., 2021. Biodecolorization of Remazol Brilliant Blue – R dye by Tropical White-Rot Fungi and their enzymes in the presence of guaiacol. *Jurnal Riset Kimia*, 12, pp.94-101.

Apriani, I., Yanto, D.H.Y., Hariani, P.L., Widjajanti, H. and Nurhayat, O.D., 2024. Potential of White Rot Fungi from Berbak-Sembilang National Park, Indonesia for decolorization and detoxification commercial Direct Dyes. *Trend in Sciences*, 21(6), pp.1-12.

Asgher, M., Muzammil, M., Ahmad, S., Khalid, N. and Bilal, M., 2020. Environmentally friendly color stripping of solar golden yellow R dyed cotton fabric by ligninolytic consortia from *Ganoderma lucidum* IBL-05. *Case Studies in Chemical and Environmental Engineering*, 2, p.100031.

Bankole, P.O., Adeyinka, A., Folashade, O., Vinayak, V. and Prabhu, S., 2018. Biodegradation and detoxification of Scarlet RR dye by a newly isolated filamentous fungus, *Peyronellaea prosopidis*. *Sustainable Environment Research*, 28, pp.214-222.

Bilal, M., Adeel, M., Rasheed, T., Zhao, Y. and Iqbal, M.N., 2019. Emerging contaminants of high concern and their enzyme-assisted biodegradation – A review. *Environment International*, 124, pp.336-353.

Bittencourt, G.A., Vandenberghe, L.P. de S., Martínez-Burgos, W.J., Valladares-Diestra, K.K., Murawski de Mello, A.F., Maske, B.L., Brar, S.K., Varjani, S., de Melo Pereira, G.V. and Soccol, C.R., 2023. Emerging contaminants bioremediation by enzyme and nanozyme-based processes – A review. *iScience*, 26(6), pp.1-19.

Castillo-M., L.A., Ramírez-G, C.A., Martínez-C, A., Reyes-G, J.R., Sánchez-O, E.M., Ibarra-V, J.R., Jiménez-J, R., Cuevas-V, G.M., Navarrete-M, A., Sandoval-A, O.A. and Gómez-R, C., 2023. Mathematical modeling for operative improvement of the decoloration of Acid Red 27 by a novel microbial consortium of *Trametes versicolor* and *Pseudomonas putida*: A multivariate sensitivity analysis. *Heliyon*, 9(11), p.e21793.

Chaturvedi, S., 2019. Azo Dyes Decolorization using White Rot Fungi. *Research & Reviews: Journal of Microbiology and Biotechnology*, 8(2), pp.9-19.

Chaurasia, P.K. and Bharati, S.L., 2019. Recent Myco-dye decolorization studies (Mini-review). *Journal of Biotechnology and Bioengineering*, 3(4), pp.27-31.

Cheng, W.N., Sim, H.K., Ahmad, S.A., Syed, M.A., Shukor, M.Y. and Yusof, M.T., 2016. Characterization of an azo-dye-degrading White Rot Fungus isolated from Malaysia. *Mycosphere*, 7(5), pp.560-569.

Colla, I.M., de Oliveira Filho, O.B.Q., de Freitas, J.D.S., Bertéli, M.B.D., Linde, G.A., Do Valle, J.S. and Colauto, N.B., 2020. Mycelial biomass cultivation of *Lentinus crinitus*. *Bioscience Journal*, 36(6), pp.2238-2246.

Coria-Oriundo, L.L., Battaglini, F. and Wirth, S.A., 2021. Efficient decolorization of recalcitrant dyes at neutral/alkaline pH by a new bacterial laccase-mediator system. *Ecotoxicology and Environmental Safety*, 217, p.112237.

Daniel, W., Schneider, H., Claudete, R., Mendonça, S., Gonçalves, F., Siqueira, D., José, A., Dillon, P. and Camassola, M., 2018. High level production of laccases and peroxidases from the newly isolated White-Rot Basidiomycete *Marasmiellus palmivorus* VE111 in a stirred-tank bioreactor in response to different carbon and nitrogen sources. *Process Biochemistry*, 69, pp.1-11.

Dao, A.T.N., Smits, M., Dang, H.T.C., Brouwer, A. and de Boer, T.E., 2021. Elucidating fungal *Rigidoporus* species FMD21 lignin-modifying enzyme genes and 2,3,7,8-tetrachlorodibenzo-p-dioxin degradation by laccase isozymes. *Enzyme and Microbial Technology*, 147, p.281-288.

Das, A., Bhattacharya, S., Panchanan, G., Navya, B.S. and Nambiar, P., 2016. Production, characterization and Congo red dye decolourizing efficiency of a laccase from *Pleurotus ostreatus* MTCC 142 cultivated on co-substrates of paddy straw and corn husk. *Journal of Genetic Engineering & Biotechnology*, 14(2), pp.281-288.

Das, S., Cherwoo, L. and Singh, R., 2023. Decoding dye degradation: Microbial remediation of textile industry effluents. *Biotechnology Notes*, 4, pp.64-76.

Dulay, R.M.R., Cabrera, E.C., Kalaw, S.P. and Reyes, R.G., 2021. Optimization of culture conditions for mycelial growth and fruiting body production of naturally-occurring Philippine mushroom *Lentinus swartzii* Berk. *Journal of Applied Biology and Biotechnology*, 9(3), pp.17–25.

Duran, D., Suspes, D., Maestre, E., Alfaro, M., Perez, G. and Pisabarro, A.G., 2022. Effect of Nutritional Factors and Copper on the Regulation of Laccase Enzyme Production in *Pleurotus ostreatus*. *Journal of Fungi*, 8, pp.1–21.

Rao, G.R., Ravichandran, A., Kandalam, G., Kumar, S.A., Swaraj, S. and Sridha, M., 2019. Enhanced production of manganese peroxidase from *Clitopilus scyphoides* employing statistical optimization for application in improving crop residue digestibility by ruminants. *Journal of Dairy, Veterinary & Animal Research*, 8(5), pp.190–203.

Hamad, H.O., Alma, M.H., Ismael, H.M. and Eri, A.G.O.C., 2014. The effect of some sugars on the growth of *Aspergillus niger*. *KSU Journal of Natural Sciences*, 17, pp.7–11.

He, X., Song, C., Li, Y., Wang, N., Xu, L., Han, X. and Wei, D., 2018. Efficient degradation of azo dyes by a newly isolated fungus *Trichoderma tomentosum* under non-sterile conditions. *Ecotoxicology and Environmental Safety*, 150, pp.232–239.

Herath, I.S., Udayanga, D., Jayasanka, D.J. and Hewawasam, C., 2024. Textile dye decolorization by white rot fungi – A review. *Bioresource Technology Reports*, 25, p.101687.

Iark, D., Buzzo, A.J.D., Garcia, J.A.A., Gesser, V., Vieira, C., Carvalho, R., Corrêa, G. and Peralta, R.A., 2019. Enzymatic degradation and detoxification of azo dye Congo red by a new laccase from *Oudemansiella canarii*. *Bioresource Technology*, 289, p.121655.

Jaramillo, A.C., Cobas, M., Hormaza, A. and Sanromán, M.Á., 2017. Degradation of adsorbed azo dye by solid-state fermentation: Improvement of culture conditions, a kinetic study, and rotating drum bioreactor performance. *Water, Air, & Soil Pollution*, 228, pp.205–219.

Jiménez, S., Velásquez, C., Mejía, F., Arias, M. and Hormaza, A., 2019. Comparative studies of pure cultures and a consortium of white-rot fungi to degrade a binary mixture of dyes by solid-state fermentation and performance at different scales. *International Biodeterioration & Biodegradation*, 145, p.104772.

Kalaw, S.P., Dulay, R.M.R., Damaso, E.J., Ramos, J.C., Del Rosario, M.A.G., Abon, M.D., De Leon, A.M., Undan, J.R. and Reyes, R.G., 2021. Optimization of mycelial culture conditions and fructification of *Lentinus* species using rice straw and sawdust based substrates. *Studies in Fungi*, 6(1), pp.519–530.

Kong, W., Chen, H., Lyu, S., Ma, F., Yu, H. and Zhang, X., 2016. Characterization of a novel manganese peroxidase from white-rot fungus *Echinodontium taxodii* 2538, and its use for the degradation of lignin-related compounds. *Process Biochemistry*, 51(11), pp.1776–1783.

Kumar, A. and Arora, P.K., 2022. Biotechnological applications of manganese peroxidases for sustainable management. *Frontiers in Environmental Science*, 10, pp.1–11.

Kumar, A. and Chandra, R., 2020. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. *Heliyon*, 6(2), p.e03170.

Kyomuhimbo, H.D. and Brink, H.G., 2023. Applications and immobilization strategies of the copper-centred laccase enzyme: a review. *Heliyon*, 9(2), p.e13156.

Laksmi, F.A., Agustriana, E., Nuryana, I., Rachmayati, R., Perwitasari, U. and Andriani, A., 2021. Removal of textile dye, RBBR, via decolorization by *Trametes hirsuta* AA-017. *Biosaintifika*, 13, pp.319–327.

Liu, S., Tsai, S., Guo, P. and Lin, C., 2020. Inducing laccase activity in white rot fungi using copper ions and improving the efficiency of azo dye treatment with electricity generation using microbial fuel cells. *Chemosphere*, 243, p.125304.

Mendes, N., Paula, D., Brugnari, T., Windson, C., Haminiuk, I. and Maria, G., 2022. Biotechnological potential of fungi from a mangrove ecosystem: Enzymes, salt tolerance and decolorization of a real textile effluent. *Microbiological Research*, 254, p.126899.

Monga, D., Kaur, P. and Singh, B., 2022. Microbe mediated remediation of dyes, explosive waste and polyaromatic hydrocarbons, pesticides and pharmaceuticals. *Current Research in Microbial Sciences*, 3, p.100092.

Noman, E., Talip, B.A., Al-Gheethi, A., Mohamed, R. and Nagao, H., 2020. Decolourisation of dyes in greywater by mycoremediation and mycosorption process of fungi from peatland; primary study. *Materials Today: Proceedings*, 31, pp.23–30.

Omar, S.A., 2016. Decolorization of different textile dyes by isolated *Aspergillus niger*. *Journal of Environmental Science and Technology*, 9(1), pp.149–156.

Park, M.S., Yoo, S., Cho, Y., Park, K.H., Kim, N.K., Lee, H.S. and Lim, Y.W., 2021. Investigation of the fungal diversity of the Federated States of Micronesia and the construction of an updated fungal inventory. *Mycobiology*, 49(6), pp.551–558.

Pazarlioglu, N.K., Akkaya, A., Akdogan, H.A. and Gungor, B., 2010. Biodegradation of Direct Blue 15 by free and immobilized *Trametes versicolor*. *Water Environment Research*, 82(7), pp.579–585.

Pérez-Cadena, R., García-Esquivel, Y., Castañeda-Cisneros, Y.E., Serna-Díaz, M.G., Ramírez-Vargas, M.R., Muro-Urista, C.R. and Téllez-Jurado, A., 2020. Biological decolorization of Amaranth dye with *Trametes polyzona* in an airlift reactor under three airflow regimes. *Heliyon*, 6(12), p.e05857.

Permpornsakul, P., Prasongsuk, S., Lotrakul, P., Eveleigh, D.E., Kobayashi, D.Y., Imai, T. and Punnapayak, H., 2016. Biological treatment of Reactive Black 5 by resupinate white rot fungus *Phanerochaete sordida* PBU 0057. *Polish Journal of Environmental Studies*, 25(3), pp.1167–1176.

Rajhans, G., Barik, A., Sen, S.K. and Raut, S., 2021. Degradation of dyes by fungi: an insight into mycoremediation. *BioTechnologia (Poznan)*, 102, pp.445–455.

Ridtibud, S., Suwannasai, N., Sawasdee, A., Champreda, V., Phosri, C., Sarp, S., Pisutpaisal, N. and Boonyawanich, S., 2024. Selection of white-rot fungi for decolorization of palm oil mill effluent and evaluation of biodegradation and biosorption processes. *Nature Environment and Pollution Technology*, 23(1), pp.235–243.

Sen, S.K., Raut, S., Bandyopadhyay, P. and Raut, S., 2016. Fungal decolouration and degradation of azo dyes: A review. *Fungal Biology Reviews*, 30(3), pp.112–133.

Sen, S.K., Raut, S. and Raut, S., 2023. Mycoremediation of anthraquinone dyes from textile industries: a mini-review. *Biotechnologia*, 104(1), pp.85–91.

Senthilkumar, S., Perumalsamy, M. and Prabhu, H.J., 2014. Decolourization potential of white-rot fungus *Phanerochaete chrysosporium* on synthetic dye bath effluent containing Amido black 10B. *Journal of Saudi Chemical Society*, 18(6), pp.845–853.

Shindhal, T., Rakholiya, P., Varjani, S., Pandey, A., Hao, H., Guo, W., Ng, H.Y. and Taherzadeh, M.J., 2021. A critical review on advances in the practices and perspectives for the treatment of dye industry wastewater. *Bioengineered*, 12(1), pp.70-78.

Singh, A.K., Iqbal, H.M.N., Cardullo, N., Muccilli, V., Fernández-Lucas, J., Schmidt, J.E., Jesionowski, T. and Bilal, M., 2023. Structural insights, biocatalytic characteristics, and application prospects of lignin-modifying enzymes for sustainable biotechnology. *International Journal of Biological Macromolecules*, 242(3), p.124968.

Singh, M.P., Vishwakarma, S.K. and Srivastava, A.K., 2013. Bioremediation of direct blue 14 and extracellular ligninolytic enzyme production by White Rot Fungi: *Pleurotus* Spp. *BioMed Research International*, 2013, pp.2–5.

Subedi, K., Basnet, B.B., Panday, R., Neupane, M. and Tripathi, G.R., 2021. Optimization of growth conditions and biological activities of Nepalese *Ganoderma lucidum* Strain Philippine. *Advances in Pharmacological and Pharmaceutical Sciences*, 2021, pp.1–7.

Sudhaparimala, S. and Usha, R., 2022. Tuning of carbon microspheres and graphene structures with hetero atoms for organic dye degradation and heavy metal remediation - influence of fructose as a precursor. *Nature Environment and Pollution Technology*, 21(2), pp.469–480.

Sukarta, I.N., Ayuni, N.P.S. and Sastrawidana, I.D.K., 2021. Utilization of khamir (*Saccharomyces cerevisiae*) as adsorbent of remazol red rb textile dyes. *Ecological Engineering & Environmental Technology*, 22(1), pp.117–123.

Sun, S., Liu, P. and Ullah, M., 2023. Efficient azo dye biodecolorization system using lignin-Co-cultured White-Rot Fungus. *Journal of Fungi*, 9(1).

Suryadi, H., Judono, J.J., Putri, M.R., Eclessia, A.D., Ulhaq, J.M., Agustina, D.N. and Sumiati, T., 2022. Biodelignification of lignocellulose using ligninolytic enzymes from White-Rot Fungi. *Heliyon*, 8(2), p.e08865.

Vel, C., Mejía-Ot, F. and Hormaza-Anaguano, A., 2020. Evaluation of several white-rot fungi for the decolorization of a binary mixture of anionic dyes and characterization of the residual biomass as potential organic soil amendment. *Journal of Environmental Management*, 254, p.109805.

Vinotha, S. and Rose, A.L., 2023. Comparative advanced oxidation decolorization of the triphenylmethane dye with dimethyl dioxirane and hydrogen peroxide. *Nature Environment and Pollution Technology*, 22(2), pp.803–815.

Wang, N., Chu, Y., Wu, F., Zhao, Z. and Xu, X., 2017. Decolorization and degradation of Congo red by a newly isolated white rot fungus, *Ceriporia lacerata*, from decayed mulberry branches. *International Biodeterioration & Biodegradation*, 117, pp.236–244.

Yanto, D.H.Y., Guntoro, M.A., Nurhayat, O.D., Anita, S.H., Oktaviani, M., Ramadhan, K.P., Pradipta, M.F. and Watanabe, T., 2021. Biodegradation and biodetoxification of batik dye wastewater by laccase from *Trametes hirsuta* EDN 082 immobilised on light expanded clay aggregate. *3 Biotech*, 11(5), pp.1–13.

Yuan, H., Li, J., Pan, L., Li, X., Yuan, Y., Zhong, Q. and Wu, X., 2022. Ecotoxicology and environmental safety particulate toxicity of metal-organic framework UiO-66 to white rot fungus *Phanerochaete chrysosporium*. *Ecotoxicology and Environmental Safety*, 247, p.114275.

Yusuf, F., Yakasai, H.M., Usman, S., Muhammad, J.B., Yaú, M., Jagaba, A.H. and Shukor, M.Y., 2023. Dyes-decolorizing potential of fungi strain BUK_BCH_BTE1 locally isolated from textile industry effluents: characterization and LC-MS analysis of the metabolites. *Case Studies in Chemical and Environmental Engineering*, 8, p.100453.

Zafar, S., Bukhari, D.A. and Rehman, A., 2022. Azo dyes degradation by microorganisms – An efficient and sustainable approach. *Saudi Journal of Biological Sciences*, 29(12), p.103437.

Zhu, C., Bao, G. and Huang, S., 2016. Optimization of laccase production in the white-rot fungus *Pleurotus ostreatus* (ACCC 52857) induced through yeast extract and copper. *Biotechnology and Biotechnological Equipment*, 30(2), pp.270–276.