

## Biodegradation of Turquoise Blue Textile Dye by Wood Degrading Local Fungi Isolated from a Plantation Area

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### ABSTRACT

This research aimed to investigate the potential screening of wood degrading fungi to degrade the textile dye. Nine fungi were selected based on their ability to remove the turquoise blue textile dye on liquid potatoes dextrose medium. The fungi which have a high color removal efficiency were identified and analyzed qualitatively in terms of the manganese peroxidase and lignin peroxidase enzymes and their enzyme activity was determined. The selected fungi were investigated for their ability to degrade turquoise blue under variations in pH, dye concentration, and contact time. The results showed that three of the nine strains of wood-degrading local fungi, identified as *Trametes hirsuta*, *Microporus xanthopus*, and *Ganoderma applanatum*, were able to degrade textile dye. The color removal efficiency of dye treatment was monitored under the initial pH, dye concentration, and contact time variation. The study analyzed that the optimal color removal efficiency of the turquoise blue textile dye with a concentration of 30 mg/L in an aqueous solution was achieved at 78.50 and 85.84% at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*, while the color removal efficiency was 82.17% at pH 5 for *Trametes hirsuta* with seven days of incubation time.

**Keywords:** Ligninolytic enzyme, removal efficiency, turquoise blue dye, wood degrading fungi.

### INTRODUCTION

In the fabric dyeing process, large amounts of wastewater containing various toxic compounds, such as heavy metals, caustic soda, and dyes, are produced. About 10 to 15% of the total dye used is lost during the dyeing process and released into the environment as wastewater [Nam and Renganathan, 2000; Wilkolazka et al., 2002]. The discharge of these effluents into receiving water bodies can cause negative environmental effects. The presence of color in water bodies causes a reduction in environmental aesthetics. It can inhibit light penetration into the water, produce the various amines compounds, and unpleasant odor

products under anaerobic conditions [Slokar and Lemarechal, 1998]. In addition, some dyes, commonly used in the fabric dyeing process are known to be toxic, carcinogenic, and mutagenic. Hence, they are harmful to aquatic life, as well as human life. Among the dyes, turquoise blue dye, a well-known reactive dye, is often used in the dyeing process and produces a dark blue color in wastewater effluent. A study by Xu et al. in 2010 found that the azo dyes have a carcinogenic effect on the liver and bladder of mammals, and are potentially mutagenic in humans. A bladder tumor has been reported more frequently for the workers in the dye industry than in general society [Suryavathi et al., 2005]. Therefore, the pollutants in textile

wastewater must be removed before discharging into water bodies.

Generally, the methods used for color removal and other contaminants in textile wastewater involve various combinations of chemical, physical and biological methods. Several physicochemical methods have been applied for textile wastewater treatment, such as electrochemical oxidation [Sastrawidana et al., 2018], adsorption using chitin-based adsorbent [Wang et al., 2020], and *Khamir* (*Saccharomyces cerevisiae*) as adsorbent of Remazol Red RB textile dyes [Sukarta et al., 2021], and photocatalytic method using  $\text{TiO}_2/\text{UV}$ -irradiation [Azzaz et al., 2021]. The biodegradation methods used for textile wastewater treatment included microalgae [Daneshvar et al., 2007; El-Sheekh et al., 2018; Premaratne et al., 2021], bacterial consortia attached growth reactor [Iqbal et al., 2022], and fungi [Dewi et al., 2018; Salem et al., 2019; Mahmood et al., 2021]. Among these methods, the biological method, mainly using wood-degrading fungi, has attracted much attention as one of the efficient techniques for removing color from dyes due to their environmentally friendly, cost-competitive, easy operation, and high color removal efficiency. Degradation of dyes by fungi is associated with the ability of fungi to produce extracellular oxidizing enzymes, such as ligninolytic extracellular enzymes, including manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase. The ligninolytic enzymes have been widely applied in food, paper, and textile [Canas and Camarero, 2010]. Fungi use extracellular enzymes to transform aromatic compounds, such as lignin, dyes, and polycyclic aromatic hydrocarbons into less toxic products [Yesilada et al., 2003]. Several strains of fungi have been reported to degrade the color of textile wastewater, including laccase enzymes-producing soil fungi of *Aspergillus niger* and *Phanerochaete chrysosporium* [Rani et al., 2014], *Emmia latemarginata*, *Mucor circinelloides*, *Aspergillus fumigatus*, *Cryptococcus spp.*, as well as *Candida albican* which were isolated from a textile industry effluent [Olufunke et al., 2016; Juárez-Hernández et al., 2021], and *Trametes polyzona* [Pérez-Cadena et al., 2020]. Basidiomycetous class fungi generally grow on rotten wood and produce extracellular ligninolytic enzymes, such as lignin peroxidase (LiP), and manganese peroxidase (MnP), laccase, versatile peroxidase (VP), and other accessory enzymes [Illuri et al., 2021]. The fungi from the Basidiomycetes class

can degrade various dye structures, such as anthraquinone, triphenylmethane, and azo dyes [Machado et al., 2005].

The main objective of the present study was to investigate and select the wood degrading local fungi that grow on wood decayed pieces located in the Buleleng-Bali plantations area for color removal of textile dye. The selected wood degrading fungi were further investigated for their ability to remove the color of textile dyes under various conditions of initial pH, dye concentration, and incubation time.

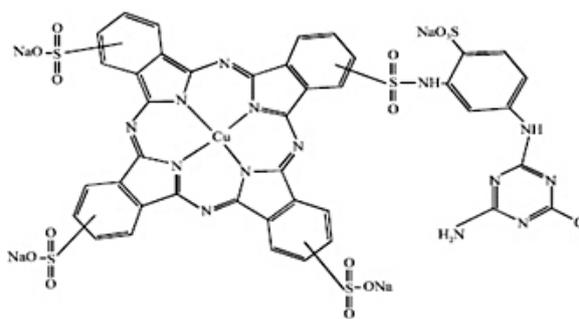
## MATERIALS AND METHODS

### Dye

The turquoise blue textile dye used in this study was obtained from Sigma Chemical Company. This dye has the molecular formula  $\text{C}_{40}\text{H}_{25}\text{CuN}_9\text{O}_{14}\text{S}_5$  with a molecular structure, as shown in Figure 1.

### Sampling and screening of wood degrading local fungi

In this study, nine local fungi growing on rotting wood pieces of tropical plants in the Buleleng-Bali area were investigated for their ability to degrade textile dye. Each fungus culture was placed in a plastic bag and then kept in a freezer at  $0^\circ\text{C}$ . The nine wood degrading local fungi were selected by growing them in Potatoes Dextrose broth containing 10 mg/L of turquoise textile dye and then incubated for 10 days at room temperature while shaking at 150 rpm using an automatic shaker. The color removal of dye was measured by a double beam Shimadzu-1700 UV-Visible spectrophotometer at 624.5 nm.



**Figure 1.** Chemical structure of the turquoise blue dye

## Identification of selected wood degrading local fungi

Three of the nine wood degrading fungi with high potential to degrade textile dye were identified based on their macroscopic and microscopic characteristics. The three selected fungi have distinctive characteristics, such as pores on the lower cap, and the presence of pores indicates the fungi class of Basidiomycetes. The macroscopic and microscopic characteristics of three wood degrading local fungi were presented in Table 1.

## Qualitative analysis of ligninolytic enzymes

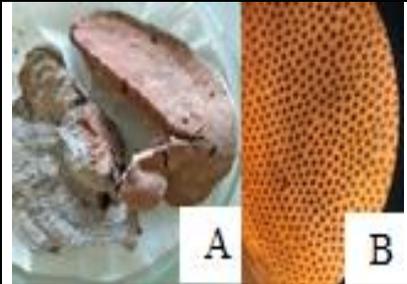
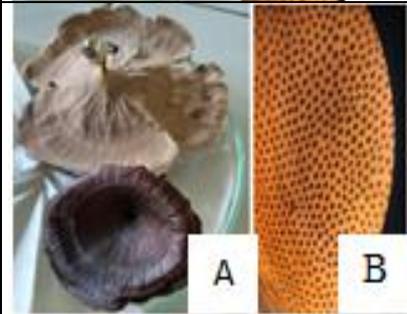
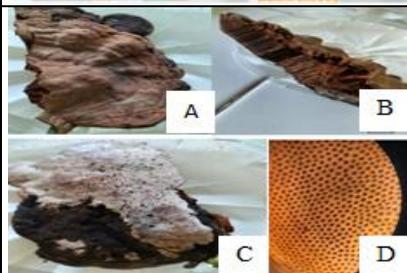
Manganese peroxidase (MnP) and lignin peroxidase (LiP) produced by wood degrading fungi were qualitatively analyzed; 2.00 mL of the culture filtrate was placed in the test tube and added with 1 mL of 1 mM  $MnSO_4$ , 0.3 mL of 1 mM phenol red, 1 mL phosphate buffer (pH 5), and 1 mL of 50 mM  $H_2O_2$  to investigate the manganese peroxidase enzyme production. The formation of a brownish orange color indicates

a positive presence of the manganese peroxidase enzyme. During the experiment, 2.00 mL of the culture filtrate was placed in the test tube and then added with reagents comprised of 1 mL of 100 mM n-propanol, 1 mL of 250 mM tartaric acid, and 1 mL of 50 mM  $H_2O_2$  to assess the lignin peroxidase enzyme production, the formation of a yellowish white color is positive for the lignin peroxidase enzyme.

## Ligninolytic enzymes activity assay

The manganese peroxidase (MnP) and lignin peroxidase (LiP) activities were assayed using a double beam Shimadzu-1700 UV-Visible spectrophotometer. The MnP activity was determined using phenol red as a substrate in the presence of  $H_2O_2$ , according to Geethanjali and Jayashankar, 2017. The oxidized phenol red was observed by reading the absorbance at 610 nm. The LiP activity was assayed using n-propanol as the substrate in the presence of  $H_2O_2$  modified from Shanmugam et al. 1999. The formation of propanaldehyde was

**Table 1.** Macro and microscopic characteristics of the wood degrading fungi

Isolate	The results of observations on the macroscopic and microscopic properties of wood-degrading local fungi	Name of fungi
	The cap is quite thick (2.5–3 cm), with a hard texture like wood, known as bracket shaped fungi, with a white surface/cap color and a clean pinky color at the bottom. At the bottom of the cap, there are medium-sized pores, as shown in Figure B.	<i>Trametes hirsuta</i>
	Main characteristics: hard fruiting bodies like wood but thin texture like paper. These fungi resist living in a dry environment by attaching to wood stems as their hosts. On the cap, there are concentric circles/lines or radial lines surrounding the cap. The bottom cap is cream/light brown and observed using a stereomicroscope. There are pores, as shown in Figure B.	<i>Microporus xanthopus</i>
	The main characteristics: are having a hard fruit body like wood (A), a hard hood surface, and brown (A, B) with a white bottom cap color (C). At the bottom of the cap, there are pores as a place for spores of these fungi (D)	<i>Ganoderma applanatum</i>

monitored at 310 nm in the reaction mixtures containing 10 mM n-propanol, 250 mM tartaric acid, and 1 mM H<sub>2</sub>O<sub>2</sub>. All the enzyme activity assays were run in triplicate and compared with reference blanks containing all the reagents except an enzyme. One unit of enzyme activity was defined as the amount of enzyme consumed to oxidize 1 μmol of substrate per minute (de Oliveira et al., 2009). The MnP and LiP enzyme activity (Uml<sup>-1</sup>) was calculated using the formula:

$$EA(U\text{ mL}^{-1}) = \frac{(A \times V)}{t \times \epsilon \times v} \quad (1)$$

where:  $EA$  = enzyme activity (U/mL),  $A$  = absorbance,  $V$  = total volume of reaction mixture (mL),  $t$  = incubation time (min),  $\epsilon$  = extinction coefficient (M<sup>-1</sup>cm<sup>-1</sup>) and  $v$  = volume of enzyme (mL). The value of  $\epsilon$  for the oxidized phenol red is 22 mM<sup>-1</sup>cm<sup>-1</sup>, while 20 mM<sup>-1</sup>cm<sup>-1</sup> for monitoring the formation of propanaldehyde.

### The color removal efficiency of turquoise blue textile dye by wood degrading fungi

Different strains of three selected wood-degrading fungi species: *Trametes hirsuta*, *Microporous xanthopus*, and *Ganoderma applanatum*, were separately investigated for their performance in degrading turquoise blue textile dye on Czapek media. A total of 50 mL of remazol turquoise blue dye with a concentration of 100 mg/L was transferred to Erlenmeyer in 100 mL and 3 mL of fungal spore suspension was added. Then, the mixture in Erlenmeyer was covered with aluminum foil and incubated for 10 days while shaking using an automatic shaker with a rotation speed of 150 rpm. After the degradation process, the mixtures were filtered through Whatman No.1 paper filter, and the supernatant was centrifuged at 3000 rpm for ten minutes. The absorbance of remazol turquoise blue before and after treatment was measured using a double beam Shimadzu-1700 UV-Visible spectrophotometer at 624.5 nm. The effect of initial pH, dye concentration, and contact time on color removal of turquoise blue textile dye was investigated, and each treatment was replicated three times. The color removal efficiency was calculated using the formula:

$$\text{Color removal}(\%) = \frac{(A_{\text{initial}} - A_{\text{final}})}{A_{\text{final}}} \times 100\% \quad (2)$$

where:  $A_{\text{initial}}$  and  $A_{\text{final}}$  are the initial and final absorbance of the turquoise blue

solution before and after days of growth, respectively.

## RESULTS AND DISCUSSIONS

### Screening of wood degrading local fungi

The nine isolates of wood degrading local fungi were collected from decaying wood in the plantation area located in Buleleng - Bali. Further, each fungus was assayed for its ability to degrade turquoise-blue textile dye. The visual appearance of the nine types of fungi is presented in Figure 2, while the color removal of turquoise blue textile dye by nine fungi cultures is shown in Figure 3.

Figure 3 shows that the nine wood degrading fungi obtained could degrade turquoise textile dye. However, it was found that three of the nine fungal strains produced the highest color removal efficiency, namely *Trametes hirsuta* (78.40%), (81.25%), and *Ganoderma applanatum* (84.10%). The strains of *Trametes hirsuta* and *Ganoderma applanatum* were obtained from decayed pieces of teak wood, while *Microporous xanthopus* came from the decay of melinjo wood.

### Qualitative test of Ligninolytic enzyme from wood degrading fungi

MnP and LiP enzymes are extracellular enzymes produced by wood-degrading fungi. These two enzymes play an important role in the degradation of turquoise blue textile dye. MnP enzymes can be tested qualitatively using phenol red and H<sub>2</sub>O<sub>2</sub> reagents, while LiP enzymes are tested using n-propanol reagents in the presence of H<sub>2</sub>O<sub>2</sub>. The qualitative analysis result of the MnP and LiP enzymes from wood-degrading fungi were presented in Figure 4.

As shown in Figure 4, the three types of wood degrading local fungi, namely *Trametes hirsuta*, *Microporous xanthopus*, and *Ganoderma applanatum*, were able to secrete extracellular enzymes, Manganese peroxidase and lignin peroxidase. In the MnP enzyme assay, the formation of brownish yellow color was due to the oxidation of phenol red by MnP enzyme in the presence of hydrogen peroxide. The oxidation of n-propanol in the presence of hydrogen peroxide by lignin peroxidase enzyme forms the color yellowish-white of propionaldehyde. These two enzymes play a significant role in degrading the turquoise blue dye solution.

No	Wood degrading fungi	No	Wood degrading fungi	No	Wood degrading fungi
1	 White fungi from teak wood	4	 Brown fungi from melinjo wood	7	 Brown fungi from teak wood
2	 Brown fungi from clove wood	5	 Brown fungi from coffee wood	8	 White fungi from wani wood
3	 Brown fungi from cotton wood	6	 Brown fungi from jackfruit wood	9	 White fungi from dapidap wood

Figure 2. Wood degrading fungi from the Balinese plantation

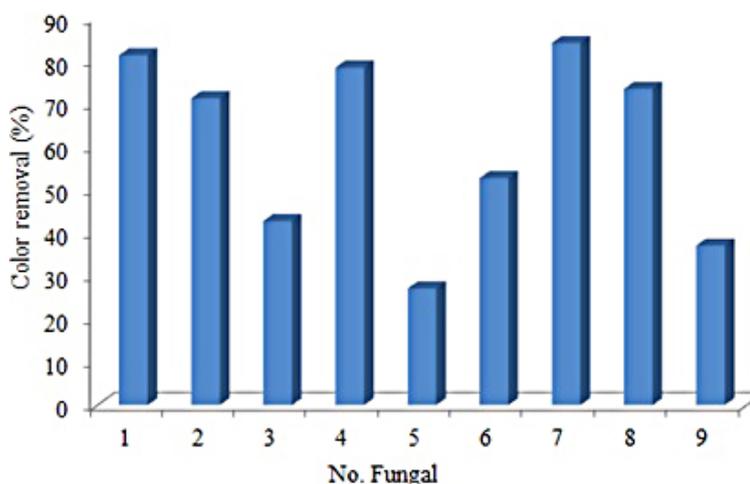


Figure 3. Color removal of turquoise blue by wood degrading fungi from the plantation area

### Enzymes activity from wood degrading local fungi

The manganese peroxidase and lignin peroxidase enzymes produced by wood-degrading fungi *Trametes hirsuta*, *Microporous xanthopus*, and *Ganoderma applanatum* were tested for enzyme activity and their role in dye degradation. The enzyme activity of manganese peroxidase was determined using phenol red as a substrate in the presence of H<sub>2</sub>O<sub>2</sub>. In contrast, the lignin peroxidase activity was assayed using n-propanol as the substrate in the presence of H<sub>2</sub>O<sub>2</sub>. The ligninolytic enzyme activity from wood degrading local fungi is shown in Table 2. The degradation of dye by

fungal culture is often associated with the ligninolytic enzyme activity [Selvam et al., 2003]. The conducted study monitored the enzyme activity of manganese peroxidase and lignin peroxidase after being cultured on a liquid Czapek medium for 10 days. The maximum lignin peroxidase activity from *Trametes hirsuta*, *Microporous xanthopus*, and *Ganoderma applanatum* were 0.32, 0.28, and 0.24 UmL<sup>-1</sup>, while the manganese peroxidase activity was 0.27, 0.24, and 0.35 UmL<sup>-1</sup>, respectively.

### Biodegradation of turquoise blue textile dye

Various physicochemical parameters, including initial pH medium (4, 5, 6, 7, 8, and 9), dye

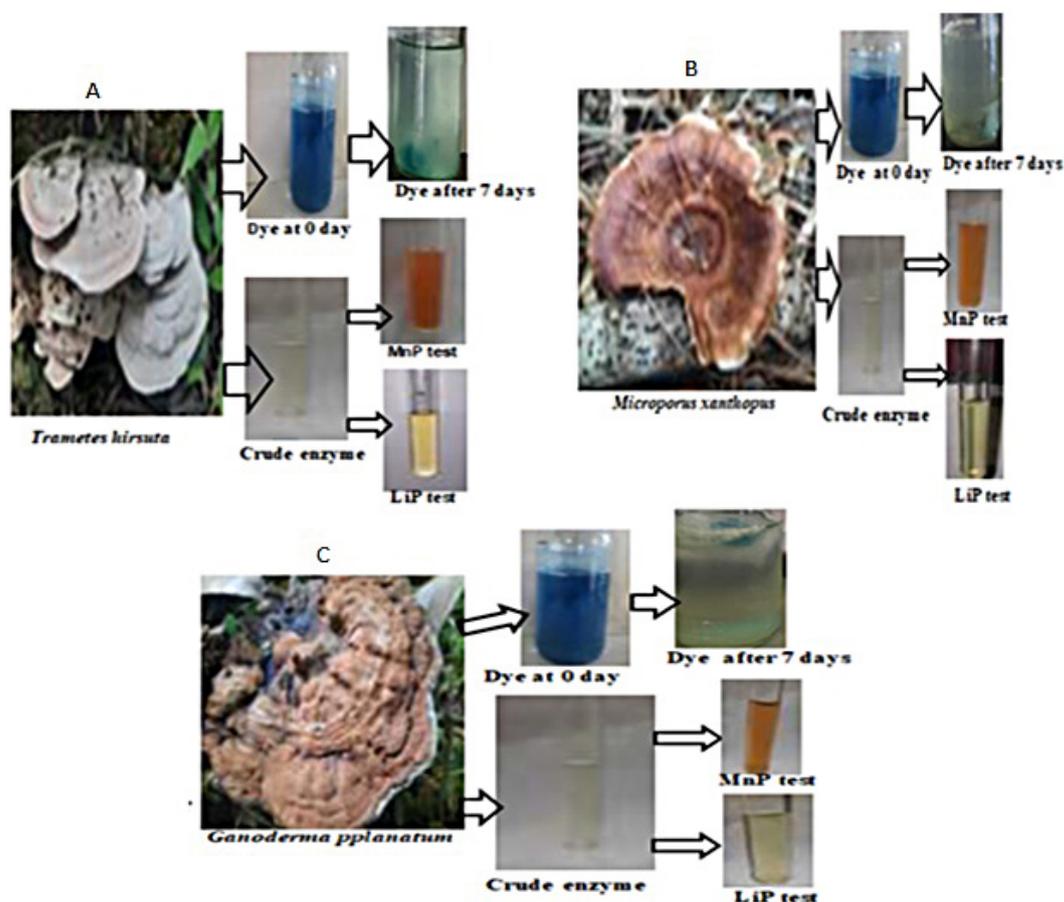


Figure 4. Qualitative test for the MnP and LiP from wood degrading fungi

Table 2. MnP and LiP activity from wood degrading fungi

Fungal	Lignin peroxidase (U <sub>mL</sub> <sup>-1</sup> )	Manganese peroxidase (U <sub>mL</sub> <sup>-1</sup> )
<i>Trametes hirsuta</i>	0.32	0.27
<i>Microporus xanthopus</i>	0.28	0.24
<i>Ganoderma applanatum</i>	0.24	0.35

concentration (10, 20, 30, 40, and 50 mg/L), and incubation time (6, 7, 8, 9 and 10 days) were investigated in terms of the color removal performance on turquoise blue textile dye by *Trametes hirsuta*, *Microporus xanthopus*, and *Ganoderma applanatum*.

### Color removal of dye at different pH of the medium

The pH of the culture media greatly affects the growth, the ability of the ligninolytic enzymes produced, and dye degradation by wood-degrading fungi. The effects of media pH on turquoise blue textile dye removal efficiency is shown in Figure 5. The result reveals that *Trametes hirsuta*, *Microporus xanthopus*, and *Ganoderma applanatum*

could decolorize turquoise blue dye over a pH range of 4.0–9.0. The optimum color removal efficiency was achieved at pH 5 for *Trametes hirsuta*. In contrast, at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*, the percent color removal at 7 days of incubation time was recorded at 85.26, 82.30, and 83.14%, respectively.

The obtained result indicates that the initial media pH is one of the important factors influencing the biodegradation process. This finding is in line with Kunjadia et al. 2020, who investigated the decolorization of azo dye from aqueous solution by ligninolytic producing fungi strain of *Pleurotus* spp. They found that the maximum color removal was obtained at pH 6. Syafiuddin and Fulazzaky, 2021, also examined the effect of

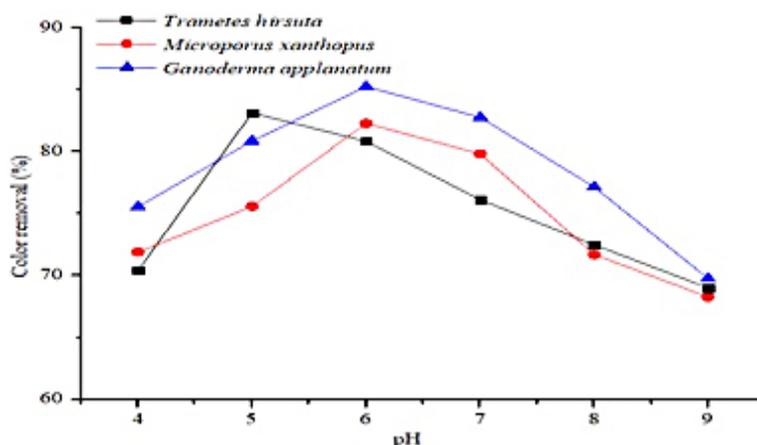


Figure 5. Color removal of turquoise blue by wood degrading fungi at different initial pH

pH on the ability of *Trichoderma citrinoviride*, *Trichoderma koningiopsis*, and *Pestalotiopsis* sp. to decolorize the Remazol Brilliant Blue R dye in aqueous solution. They suggested that the favorable pH to change the color of this dye was in the pH range of 3–5. The higher dye removal efficiency on slightly acidic media was associated with the stability of the MnP enzyme under acidic conditions [Asses et al. 2018].

#### Color removal of dye at various concentrations of dye

The color removal of turquoise blue dye by three selected wood degrading fungi was investigated in the varying dye concentration (10, 20, 30, 40, and 50 mg/L). The percent decolorization of dye by each fungus is presented in Figure 6.

On the basis of the graph in Figure 6, it was observed that the ability of three selected wood degrading fungi to decolorize turquoise blue dye was increased by the increasing dye concentration from 10–30 mg/L. However, it was decolorizing ability decreased sharply with higher dye concentration treatment. The maximum dye degradation was obtained at a dye concentration of 30 mg/L with a color removal efficiency of 78.82% and 88.22% at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*, respectively, while the color removal efficiency was 87.21% at pH 5 for *Trametes hirsuta*. Most of the previous studies also showed a decrease in the ability of fungi to degrade dyes at higher concentrations, because dyes with higher concentrations would be toxic to metabolic activity. Zhuo et al. 2011, reported that the color removal efficiency of methyl orange using white-rot

fungus decreased drastically from approximately 100% to less than 60%, when the dye concentration was increased from 25 mg/L to 100 mg/L. Laksmi et al. (2021) investigated the decolorization of Remazol Brilliant Blue Reactive dyes at different concentrations (50–200 mg/L) using *Trametes hirsuta* with the addition of 0.8 mM  $\text{CuSO}_4$  as inducers in the decolorization process. Their research revealed that the dye degradation efficiency decreased gradually with increasing dye concentration. *Trametes hirsuta* decolorized dye maximally at the dye concentration level of 50 mg/L with the color removal efficiency of 79.5% for 48 hours of incubation time.

#### Color removal of dye at different incubation times

The influence of incubation time on the degradation of the turquoise blue textile dye process was studied by varying the contact time between 6 to 10 days. The other operational conditions were 30 mg/L of dye and adjusted at pH 5 for *Trametes hirsute*, while at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*. The effect of incubation time of three selected wood degrading local fungi on the color removal efficiency of turquoise blue textile dye is shown in Figure 7.

Figure 7 illustrates that the color removal efficiency of dye by all wood degrading fungi increased from 6 to 7 days and then tended constantly from incubation time of 7 to 10 dye. The result revealed that the dye decolorization rate ranged from 78.50 to 85.84% after 7 days, while after 10 days of incubation time it ranged between 78.51 and 85.65%.

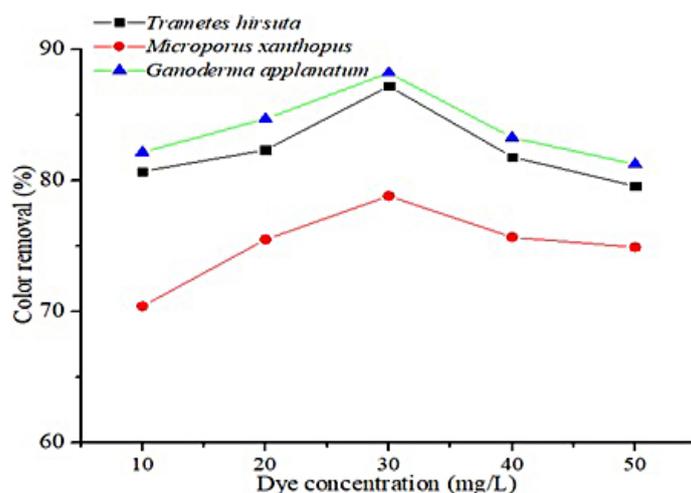


Figure 6. Color removal of turquoise blue by wood degrading fungi at a different dye concentration

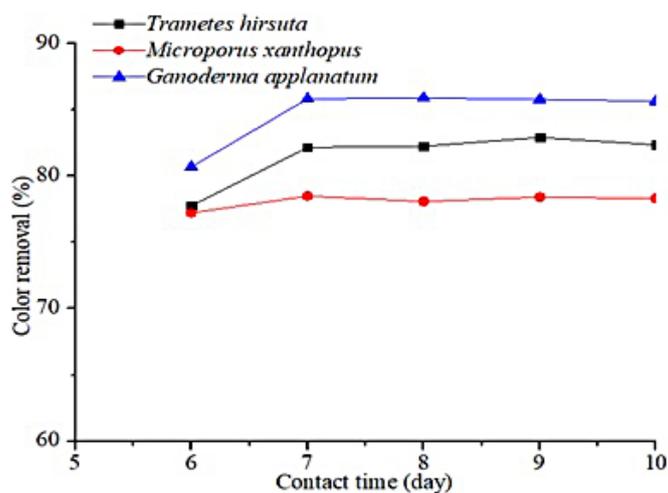


Figure 7. Color removal of turquoise blue by wood degrading fungi at different contact time

## CONCLUSIONS

On the basis of the results obtained, three local fungi, namely *Trametes hirsuta*, *Microporus xanthopus*, and *Ganoderma applanatum*, that grew on rotten wood could degrade turquoise textile dye effectively. The dye decolorization capabilities of the fungal strains are related to the extracellular excretion ability of ligninolytic enzymes, such as manganese peroxidase and lignin peroxidase. The color removal efficiency of turquoise blue textile dye by *Trametes hirsuta*, *Microporus xanthopus*, and *Ganoderma applanatum* reached 82.17, 78.50, 85.84%, respectively. The optimum operational parameters were obtained at a dye concentration of 30 mg/L, an initial pH of 5 for *Trametes hirsuta*, while at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum* with seven days of incubation time.

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