# Comparison of the Decolorization of Batik Sujo Wastewater Using Indigenous Wood Rot Fungi *Mycelioptora thermophila* KLUM1 with *Phanerochaete chrysosporium* by Solid-State Fermentation (SSF) Methods

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**Abstract.** The indigenous Wood Rot Fungi (WRF) *Mycelioptora thermophila* is known to produce ligninase enzymes and can degrade textile dyes. This study aimed to compare the adaptability, decolorization efficiency, and ligninase enzyme production of *M. thermophila* isolates with *Phanerochaete chrysosporium* on Sujo batik wastewater, also to determine the characterization of Sujo batik wastewater quality standards before and after solid-state fermentation (SSF) treatment using *M. thermophila.* Adaptability was assessed by monitoring fungal cell numbers in media with various concentrations of batik wastewater over 28 days. Decolorization and ligninase (LiP, MnP, and laccase) activity were measured during SSF using sawdust as substrate. Results showed that *M. thermophila* had superior adaptability and decolorization ability (73.6%) compared to *P. chrysosporium* (63.8%) on day 14. *M. thermophila* produced LiP (10.22 U/mL), MnP (13.33 U/mL), and laccase (8.91 U/mL), while *P. chrysosporium* showed lower MnP and laccase activity. Mycoremediation using *M. thermophila* significantly improved wastewater quality, reducing pH (from 11.6 to 6.8), BOD (3140 to 2036 mg/L), COD (11,600 to 6350 mg/L), TSS (3376 to 87.2 mg/L), total Cr (0.7 to <0.0168 mg/L), and increasing $LC_{50}$ values (28.91 to 49.93 mL/L). These results demonstrate the potential of *M. thermophila* in sustainable batik wastewater treatment.

# 1. Introduction

The batik industry is one of the creative economy sectors that continues to grow globally. Batik is not only known as the cultural heritage of Southeast Asia, but has also become a high-value textile commodity in the International market due to its unique motifs and dyeing techniques. In Indonesia, batik has been designated as a *Masterpieces of the Oral and Intangible Heritage of Humanity* by UNESCO since 2009 and has become an important part of the national cultural identity [1]. Along with increasing domestic demand and exports, batik production in Indonesia has experienced significant growth, both in quantity and distribution of production area. This increase in production has a positive impact on the local economy, creates jobs, and supports the preservation of traditional culture. However, on the other hand, the increase in demand for batik has a negative impact on the environmental ecosystem.

The batik production process uses the main materials in the form of synthetic dyes and chemicals, such as sodium silicate and batik wax, as well as high water consumption, used in high quantities in the dyeing process [2]. Almost 95% of the water after the dyeing and color decay process cannot be reused and disposed of as wastewater. As a result, batik industry wastewater containing dyes as xenobiotic compounds (compounds that are difficult to decompose and generally toxic) has a high potential to pollute the environment.

Batik Sujo is a small and medium community business that is one of the models of the Batik industry that is developing in Indonesia. According to the Center for Handicrafts and Batik, the production process of one piece of batik fabric requires 25 liters of water. Sujo's batik production capacity produces at least 30 pieces of batik fabric per month, so that every month at least 712 liters of wastewater are produced. During the production process, Batik Sujo wastewater is produced that is deep blue and is suspected to be toxic, because wastewater that is directly disposed of into the yard will kill the grass and wild plants around the waste (Interview results, 2022). Literature studies also corroborate this conjecture. Batik wastewater that is absorbed into the soil can clog soil pores and kill soil microbes so that it can reduce soil fertility [3]. Wastewater that flows into the river near the batik production process until it empties into the shore will cause changes in the physico-chemical characteristics of the aquatic ecosystem, namely pH, temperature, TSS (*Total Suspended Solids*), COD (*Chemical Oxygen Demand*), BOD (*Biochemical Oxygen Demand*). Therefore, it is very important to treat batik wastewater before it is disposed of into the environment. The processing aims to ensure that batik wastewater released into the environment is safe, characterized by meeting the parameter values set by the quality standards of textile wastewater.

Several efforts have been made to treat the wastewater of the batik industry, with physical and chemical procedures, such as adsorption, coagulation, flocculation, flotation, precipitation, oxidation, and reduction [4], [5]. However, these technologies are primarily accessible to large-scale industries due to their relatively high infrastructure and operational costs. This poses a challenge for most small-scale batik producers, especially *UMKM (micro, small, and medium enterprises)*, who typically lack waste treatment facilities. Thus, there is a clear need for a more affordable, eco-friendly, and effective alternative particularly one that focuses on color removal, a key pollutant parameter in batik wastewater.

A promising alternative to processing Batik Sujo wastewater can be done by mycoremediation technique using wood rot fungi (WRF). WRF can produce oxidative extracellular enzymes, namely manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase (Lac). These three enzymes are responsible for decomposing various xenobiotic compounds, especially polyaromatic compound derivatives found in synthetic dyes [6]. *Phanerochaete chrysosporium* is a WRF that is often referred to and studied in relation to it as a ligninase producer [7], which is a white WRF of *the Basidiomycota* group that originates

from subtropical regions [8]. *P. chrysosporium* isolates ITB was able to produce LiP with an activity of 77.4 ± 13.1 U/mg in the modified Kirk medium and was able to decolorize reactive black 5 by 80% under the experimental conditions used [9]. *M. thermophilia* KLUM1 is an indigenous WRF isolated from a cocoa plantation in Kediri, belongs to the *Ascomycetes* group, and has the ability to produce ligninase. *M. thermophila* in Kirk's modified medium is able to produce ligninase enzymes in the form of LiP, MnP, and Laccase of 4.30 U/mL, 3.30 U/mL, and 1.65 U/mL, respectively [10]. Nihayah (2018) proved that *M. thermophila* in Kirk's modified media was able to decolorize *reactive black* 5 with a percentage of 24.999%, reactive blue HEGN reached 61.895%, and rhodamine B by 10.918% [11]. Despite this potential, comparative studies between these two fungi in actual batik wastewater, particularly from local community-based industries like Batik Sujo, are still lacking. Therefore, this study aims to: characterize the properties of Batik Sujo wastewater, compare the adaptability and enzyme activity (LiP, MnP, Lac) of *P. chrysosporium* and *M. thermophila KLUM1* at varying wastewater concentrations, evaluate their decolorization capabilities, and assess the environmental impact of the mycoremediation process. The results of this study are expected to provide empirical evidence for the development of low-cost and environmentally friendly wastewater treatment strategies, particularly for small-scale batik producers in Indonesia.

## 2. Material and Methods

The indigenous wood rot fungi (WRF) *M. thermophila* KLUM1 was obtained from weathered cocoa husk waste from the Sepawon Cocoa Plantation, Kediri Regency and the isolate of *P. chrysosporium* is an ITB isolate from one of the pure culture collections of the ITB Microbiology Laboratory. The two WRF isolates were cultivated at the Biotechnology Laboratory, Biotechnology Program, Faculty Mathematics and Science State University of Malang. The sample used was wastewater from Batik Sujo UMKM, Sumberejo Village, Gedangan District, Malang Regency, East Java. The materials used in this study included *Artemia salina* larvae for toxicity testing, which were obtained from Splendid Market, Malang, with the specification *Artemia Supreme Plus Golden West* by ABK Farm Original; Potato Dextrose Agar (PDA) medium (Himedia); distilled water (aquadest); acetate buffer; and teak sawdust as a supporting medium. The production medium used is Kirk Medium, which is formulated from $NH_4(SO_4)_2$ (100 mM) as a nitrogen source, $KH_2PO_4$, $MgSO_4 \cdot 7H_2O$, $CaCl_2$ as basal medium, thiamine-HCl (100 mg/L), veratryl alcohol (3000 ppm), as well as Trace element 5X containing $MgSO_4 \cdot 7H_2O$, NaCl, $FeSO_4$, $CoCl_2$, $ZnSO_4$, $CuSO_4 \cdot 5H_2O$, $AlK(SO_4)_2$, and nitrilotriacetate as an important source of metal ions in the production of ligninolytic enzymes. Other chemicals used include NaOH 1%, $H_2SO_4$ 1%, Tween-80 0.02%, tartaric acid 0.2 M, $H_2O_2$ 50 mM, and guaiacol 10 mM.

### 1.1 Fungal strains and culture conditions

*M. thermophila* KLUM1 and *P. chrysosporium* were inoculated in a Petri dish containing Potato Dextrose Agar (PDA) medium, incubated for 24 hours at a temperature of 37ºC as stock culture.

### 1.2 Spore Suspension Preparation

All stages of spore suspension preparation were conducted aseptically. The isolates of *M. thermophila* and *P. chrysosporium* from stock culture are taken aseptically using an Enten

needle, to be inoculated into PDA slant agar, and incubated for 14 days at a temperature of 37°C. The spores formed were resuspended using a Tween-80 0.02% as much as 4 mL, left for 5 minutes, and the spores were taken by scraping using an ose needle. The resulting suspension was vortexed for 10 minutes to ensure homogenization, left to settle for 30 minutes, and filtered using a sterile syringe fitted with sterile cotton to obtain a clean spore suspension. For *M. thermophila*, the initial number of spores used per production batch was standardized at 2.10 x $10^7$ cells. The optical density (OD) of the suspension was measured at 660 nm ($OD_{660}$ = 2.81), which corresponded to a cell density of 24.4 × $10^6$ cells/mL. The required volume of spore suspension was calculated based on Equation (2), derived from Equation (1).

$$\Sigma\ cell\ density = \frac{ODmeasurement}{ODstandard} \times stand.\ cell\ density\ \left(\frac{cell}{ml}\right).\ vol\ (ml) \quad (1)$$

$$\text{Vol (mL)} = \frac{2{,}10 \times 10^7 x\ OD\ standard}{cell\ density\ x\ OD\ measured} \quad (2)$$

For *P. chrysosporium*, the spore suspension was measured at a wavelength of 600 nm ($OD_{600}$). The number of cells was determined using a hemocytometer, and the average cell count was used to estimate the standard spore density based on Equation (3), as summarized in Table 1. Notably, an $OD_{600}$ value of 0.35 corresponded to a standard cell density of 9.67 × $10^5$ cells/mL. The number of cells required for each experiment was then calculated using Equation (4), and the inoculation volume was determined using Equation (5).

$$stand.\ cell\ density\ \left(\frac{cell}{ml}\right) = \text{Z x } 10^4 \quad (3)$$

$$cell\ density = \frac{ODmeasurement}{ODstandard} \times stand.\ cell\ density\ \left(\frac{cell}{ml}\right).\ vol\ (ml) \quad (4)$$

$$V1 \text{ x M1} = \text{V2 x M2} \quad (5)$$

**Table 1.** Cell count determination

| Isolate | Standard OD600 | Standard density |
|---|---|---|
| *P. chrysosporium* | 0.35 | 9.67 x $10^5$ cell/ml |

## 1.3 Quality Analysis of Wastewater of Sujo Batik

Sujo Batik wastewater was analyzed for several parameters, including pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total chromium (Cr), and lethal concentration 50 ($LC_{50}$). The analysis followed standard procedures for wastewater quality assessment as outlined in the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.16/ME*NLHK/SETJEN/KUM.1/4/2019, a*nd referred to the Indonesian National Standard (SNI). BOD, COD, TSS, and total Cr analyses were conducted at the Water Quality Testing Laboratory of Perum Jasa Tirta. pH levels were measured using a pH meter. BOD was determined using the APHA 5210 B-2017 method. COD was analyzed following the SNI 6989.2:2019 standard, using a closed reflux method with spectrophotometry. TSS was measured using the gravimetric method, referring to APHA 2540 D-2017. Total Cr was analyzed using the APHA 3120 B, 23rd edition (2017) method. $LC_{50}$ toxicity testing was carried out using the brine shrimp lethality test (BSLT) with *Artemia salina* larvae.

### 1.4 Determination of Absorption Visible Spectrum Profile of Wastewater of Sujo Batik

The determination of the maximum wavelength ($\lambda$max) of Sujo batik wastewater was conducted to obtain the optimal absorption value for decolorization analysis. The absorption spectrum was measured using a UV-Vis spectrophotometer within a wavelength range of 300–700 nm, at 10 nm intervals. Distilled water was used as a blank, and the absorbance value was zeroed at each wavelength shift. A total of 2 mL of wastewater sample was placed in a clean cuvette, which had been rinsed with the same solution to be measured. The absorbance was then recorded at each wavelength up to 700 nm. Spectral scanning was performed on day 0 (before decolorization) and day 14 (after decolorization) for treatments with *M. thermophila* and *P. chrysosporium*, respectively. The resulting spectral data were used to determine the $\lambda$max and to evaluate changes in the absorption profile, serving as indicators of the effectiveness of the decolorization process.

### 1.5 Adaptation Test of *M. thermophila* and *P. chrysosporium* Using Media Containing Wastewater of Sujo Batik

The adaptation test began with the inoculation of *M. thermophila* and *P. chrysosporium* spore suspensions into 250 mL Erlenmeyer flasks containing 100 mL of media supplemented with different volumes of Sujo batik wastewater (0.1 mL, 1 mL, and 10 mL). Additionally, one flask contained 100 mL of wastewater only, without any production medium, to test fungal growth in undiluted wastewater conditions.The initial spore density (day 0) was determined using a hemocytometer. All Erlenmeyer flasks were incubated at 37°C for 28 days, and spore density was measured again on days 7, 14, 21, and 28 using the same method.

### 1.6 Determination of Decolorization and Ligninase Production of *M. thermophila* and *P. chrysosporium* Using the Solid-State Fermentation (SSF) Method

Decolorization of Sujo batik wastewater was carried out using the Solid-State Fermentation (SSF) method. Each 250 mL Erlenmeyer flask was prepared with 7 g of teak sawdust as a solid substrate and 10 mL of Sujo batik wastewater. This mixture was sterilized by autoclaving at 121 °C for 15 minutes. After cooling, 10 mL of pre-sterilized Kirk's medium was aseptically added to each flask. Spore suspensions of *M. thermophila* and *P. chrysosporium* were then inoculated aseptically into the flasks. A control treatment was also prepared without fungal inoculation. The cultures were incubated at 37 °C, and observations were conducted on days 0, 7, 14, 21, and 28 to assess decolorization and ligninolytic enzyme activity. At each time point, 50 mL of acetate buffer (pH 5.0) was added to stabilize the pH, followed by filtration using Whatman No. 1 filter paper. The resulting filtrate was used to measure the activities of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), as well as absorbance at 540 nm in both test and control samples. The percentage of decolorization was calculated using Equation (6).

$$Percentage\ of\ decolorization\ (\%) = \left(\frac{A_{initial} - A_{final}}{A_{initial}}\right) \times 100\% \qquad (6)$$

Where:

$A_{Final}$ = Absorbance at final time

$A_{Initial}$ = Initial absorbance at 540 nm

### 1.7 Lignin peroxidase Assay

The lignin peroxidase assay was carried out by taking 0.8 mL of veratryl alcohol 10 mM which functions as a substrate. Furthermore, 1 mL of 0.2 M of tartaric acid and 1,680 mL of distilled water were added in the test tube, and 0.2 mL of enzyme crude extract was added. The mixture was incubated for 2 minutes, then the reaction was initiated with the addition of 0.320 mL of $H_2O_2$ 5 mM. Absorbance was measured at a wavelength of 310 nm at the 0th and 1st minutes. Based on the absorbance value obtained, the activity of LiP enzyme was determined using Equation (7), the value of the molar excitation coefficient ($\varepsilon$) = 9300 $M^{-1}cm^{-1}$. The calculation of enzyme activity is by using Equation (7) below:

$$Lignin\ peroxidase\ activity\left(\frac{U}{ml}\right) = \frac{(At-A0)\times V_{tot}\times 10^6}{\varepsilon_{maks}\times d\times V_{\ enzyme}\times t} \quad (7)$$

Where:
At : Absorbance at 1st minute
A0 : Absorbance at 0 minutes
$\varepsilon_{maks}$ : molar extinction coefficient
d : Thickness of cuvette (cm)

### 1.8 Mangan peroxidase Assay

The manganese peroxidase (MnP) assay was performed by preparing a reaction mixture consisting of 0.5 mL of 0.2 M acetate buffer (pH 5.5), 0.8 mL of guaiacol solution, 1 mL of 20 mM citric acid, 0.5 mL of 50 mM $H_2O_2$, 1.5 mL of 0.1 mM $MnSO_4$, and 0.2 mL of crude enzyme extract. The mixture was incubated for 15 minutes at room temperature. MnP activity was determined by measuring the amount of oxidized guaiacol, which exhibits a color change detectable at 465 nm. Absorbance was recorded at minute 0 and minute 1 using a spectrophotometer. The enzyme activity was calculated based on the increase in absorbance, using molar extinction coefficient (ε) = 12,100 $M^{-1}\cdot cm^{-1}$, following Equation (7).

### 1.9 Laccase Activity Test

Laccase activity was measured based on its ability to oxidize guaiacol, forming a colored product detectable by spectrophotometry. The assay was conducted by mixing 0.8 mL of 10 mM guaiacol solution with 0.8 mL of 0.2 M acetate buffer (pH 5.0), followed by the addition of 0.2 mL of crude enzyme extract. The reaction mixture was transferred into a cuvette, and the absorbance was measured at 470 nm at minute 0 and minute 1 using a UV-Vis spectrophotometer. The activity of the laccase enzyme was calculated based on the increase in absorbance, using the molar extinction coefficient (ε) = 6740 $M^{-1}\cdot cm^{-1}$, according to Equation (7).

## 3. Result and Discussion

### 3.1 Quality of wastewater of Sujo Batik

The physicochemical characteristics of Sujo batik wastewater were analyzed by measuring key parameters including pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), and total Chromium (Cr). The results were compared against the wastewater quality standards set forth in the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia No. P.16/MENLHK/SETJEN/KUM.1/4/2019, as presented in Table 2. Toxicity testing was

conducted using the brine shrimp lethality test (BSLT) method, and the mortality rates of *Artemia salina* at different concentrations of wastewater are summarized in Table 3.

**Table 2.** Comparison of wastewater of Sujo Batik Wastewater Parameters with Quality Standards

| Parameters | Maximum Level (Quality Standards)* | Sujo Batik Wastewater |
|---|---|---|
| pH | 6-9 | 11,58 |
| BOD (mg/L) | 60 | 3140 |
| COD (mg/L) | 150 | 11600 |
| TSS (mg/L) | 50 | 3376 |
| Cr total (mg/L) | 1,0 | 0,7110 |

Source : *Peraturan Menteri Lingkungan Hidup, 2019 [12].

**Table 3.** Percentage of *A. salina* mortality to differences in wastewater concentrations

| Concentration (ppm) | Percentage of deaths (%) |
|---|---|
| 0 | 0 |
| 10 | 33 |
| 50 | 73 |
| 100 | 90 |

The analysis of wastewater parameters in Table 1 indicates that Sujo batik wastewater is severely polluted and exhibits high toxicity. The pH value of 11.58 classifies the effluent as strongly alkaline, exceeding the regulatory limit (6–9). This extreme pH level is likely attributable to the use of sodium silicate (waterglass) during the dye-fixation stage of batik processing. Additionally, the BOD and COD values—3,140 mg/L and 11,600 mg/L, respectively reveal a high organic and chemical pollutant load, which can significantly reduce the dissolved oxygen content in receiving water bodies. The TSS concentration was also markedly elevated (3,376 mg/L), indicating a substantial presence of particulate matter. Although the total Cr concentration (0.711 mg/L) remains below the maximum threshold, it warrants continued monitoring due to the potential contribution of heavy metals from synthetic dyes.

The acute toxicity of Sujo batik wastewater was further confirmed through BSLT analysis, with mortality rates of *A. salina* increasing in proportion to wastewater concentration (Table 2). Probit regression analysis was conducted to determine the $LC_{50}$ value, with the relationship between log wastewater concentration (x) and probit mortality (y) shown in Figure 1. The resulting regression equation, $y = 3.0968x + 0.4753$, indicates a strong positive correlation. Based on this equation, the $LC_{50}$ value was calculated to be 28.91 mL/L, assuming a parent concentration of 1,000 ppm. This finding suggests that Sujo batik wastewater is highly toxic, as even a relatively low concentration was sufficient to induce 50% mortality in the test organism.

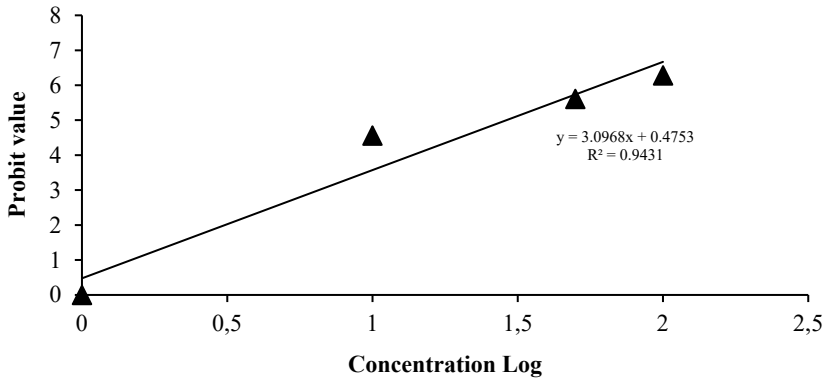


**Fig. 1.** Regression Graph of Probit Analysis of Wastewater Concentration of Batik Sujo on Mortality of *A. salina.*

### 3.2 Adaptation of *M. thermophila* and *P. chrysosporium* on Media Containing Sujo Batik Liquid Waste

The experimental results demonstrated that *M. thermophila* exhibited a greater adaptability in media containing Sujo batik wastewater compared to *P. chrysosporium* across all tested concentrations (Figure 2). At the beginning of the incubation period (day 0), both fungal isolates showed an equal spore density of $4.52 \times 10^5$ cells/mL. However, *M. thermophila* displayed a consistent increase in cell density from day 7 to day 21 at all wastewater concentrations. This increase is attributed to spore germination and subsequent hyphal proliferation facilitated by the availability of nutrients in the media. Notably, even in media containing 100 mL of wastewater without any supplemental nutrients, *M. thermophila* maintained active growth up to day 21 before a slight decline on day 28.

In contrast, *P. chrysosporium* showed limited growth, which was only observed at lower wastewater concentrations (0.1–10 mL). No significant increase in cell density was recorded in media containing 100 mL of wastewater over the 28-day incubation period. This indicates that *P. chrysosporium* may have lower tolerance to the chemical constituents or toxicity levels present in Sujo batik wastewater.

The superior adaptability of *M. thermophila* can be attributed to its thermophilic nature and its well-documented ability to thrive in harsh environmental conditions. This white-rot fungus is known for its robust extracellular enzymatic system, particularly lignin-degrading enzymes, which enables it to degrade complex aromatic and xenobiotic compounds commonly found in synthetic textile dyes. Consequently, *M. thermophila* is capable of utilizing wastewater containing dye residues as a carbon and energy source by breaking down aromatic structures into assimilable metabolites.

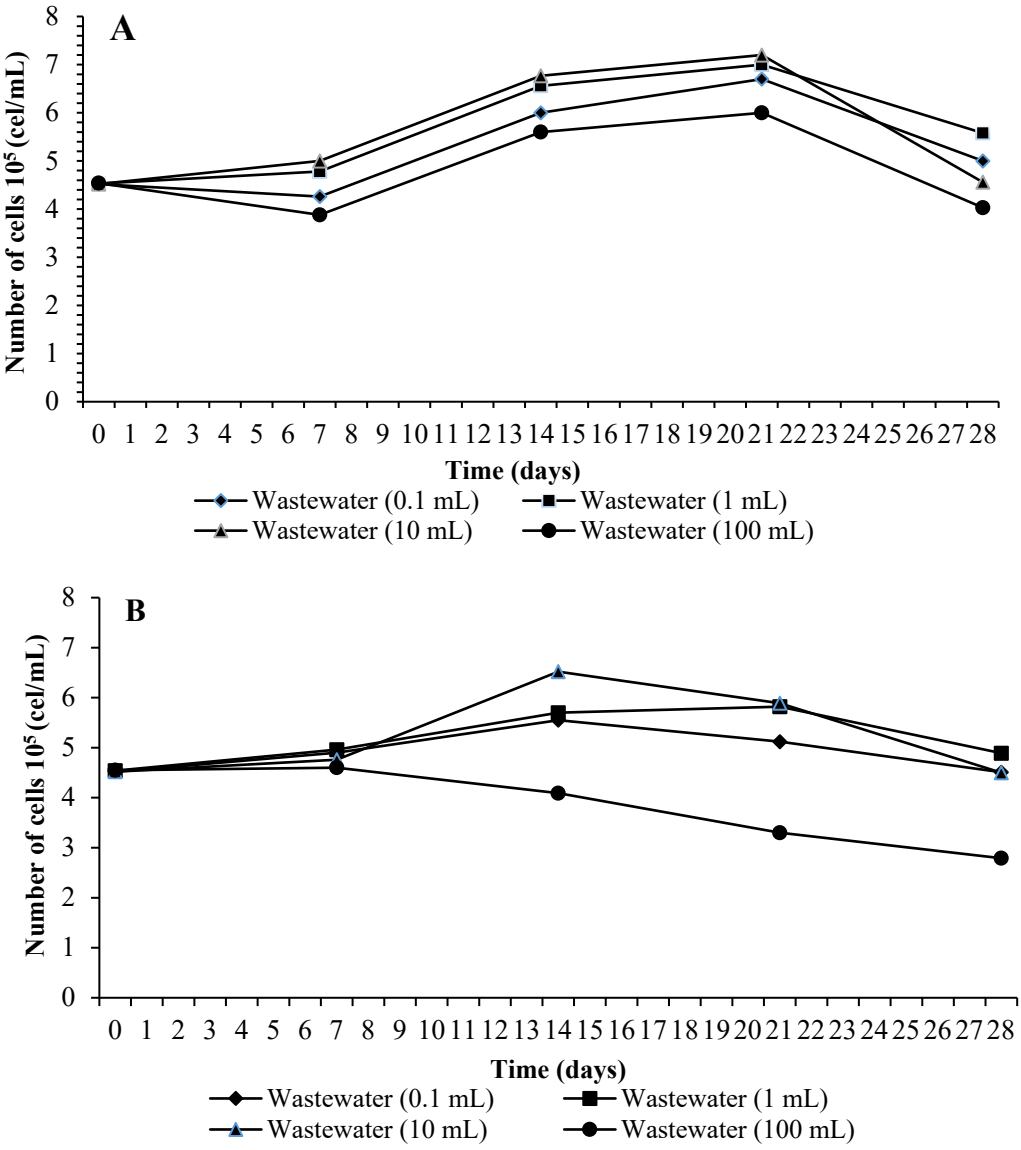


**Fig. 2.** Cell Density Comparison Graph : (A) *M. thermophila* isolate, (B) *P. chrysosporium* isolate

### 3.3 Decolorization of *M. thermophila* and *P. chrysosporium* by *Solid-State Fermentation* (SSF) Method

Previous research showed that the decolorization process of RB5 by *M. thermophila* KLUM1 was significantly enhanced by the presence of teak sawdust lignin as an alkaline carbon source in Kirk's medium. Lignin played a crucial role not only as a structural component of the solid substrate but also as an active contributor to decolorization through enzymatic degradation by ligninase, as well as adsorption mechanisms—both by the fungal mycelia and the lignin itself [13]. As shown in Figure 3, solid-state fermentation (SSF) on sawdust further supported fungal growth, with visible colonization by *M. thermophila* and *P. chrysosporium*

after 14 days of incubation. The lignin-rich sawdust provided a favorable porous matrix that facilitated oxygen diffusion and nutrient accessibility, thereby promoting fungal metabolism and enhancing decolorization performance.

The decolorization process using wood rot fungi through solid-state fermentation (SSF) on a sawdust as supporting material is depicted in Figure 3, showing the growth of *M. thermophila* and *P. chrysosporium* isolates on days 0 and 14. Visual observations indicated robust fungal growth on the sawdust substrate after 14 days of incubation, suggesting that both fungal isolates were able to effectively utilize the solid substrate as a nutrient source. Sawdust, with its high porosity, provides favorable aeration and surface area, facilitating nutrient accessibility and oxygen diffusion required for optimal fungal metabolism.

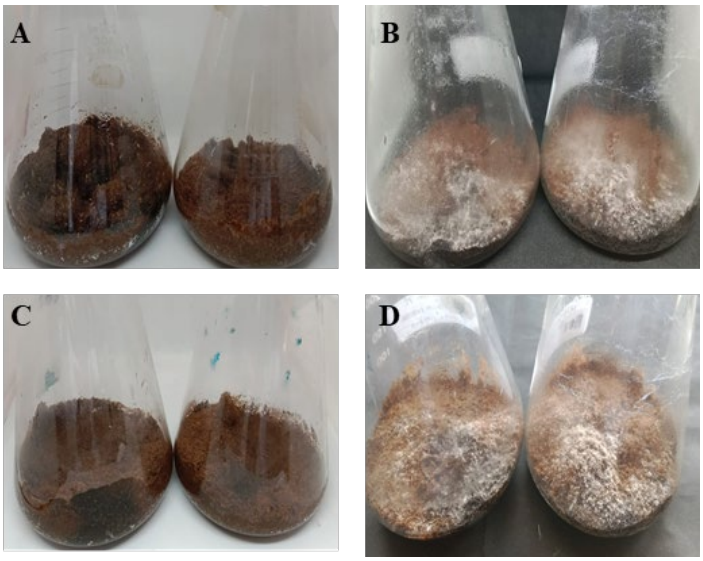


**Fig. 3.** Decolorization process by SSF method by isolate: (A) *M. thermophila* on day 0, (B) *M. thermophila* on day 14, (C) *P. chrysosporium* on day 0, (D) *P. chrysosporium* on day 14

Quantitative assessment of decolorization was carried out by measuring the absorbance of the filtrate obtained post-filtration. The results, as presented in Figure 4, demonstrate that both isolates were capable of decolorizing dyes in Sujo batik wastewater, albeit with differing efficiencies.The maximum decolorization efficiency was observed on day 14, with *M. thermophila* achieving 73.6% and *P. chrysosporium* 62.8%. After this peak, minimal changes were recorded on days 21 and 28, correlating with a decline in ligninolytic enzyme activity during the later incubation phases. The differential decolorization capacities of the two fungal isolates can be attributed to their enzymatic capabilities, physiological adaptability, and the chemical complexity of the dye compounds present. The higher the ligninolytic enzyme activity, the more efficient the degradation of chromophoric structures, which are primarily aromatic compounds responsible for color in textile dyes. Lignin-degrading enzymes (ligninases), particularly lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, catalyze the oxidation and cleavage of the aromatic rings in chromophores, leading to dye decolorization [14]. The secretion of these enzymes is highly dependent on environmental parameters such as substrate composition, nutrient availability, and oxygen levels.

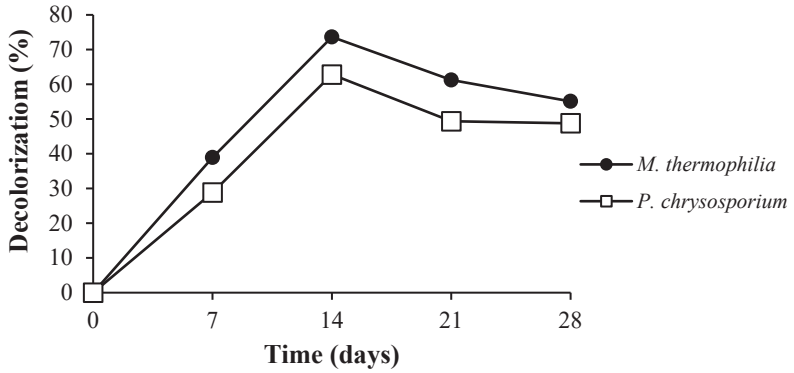


**Fig. 4.** Comparison Graph of the Decolorization Process by *M. thermophila* and *P. chrysosporium*

Comparative studies further substantiate these findings. Nihayah et al. (2018) demonstrated that *M. thermophila* was capable of decolorizing a range of dyes including methylene blue, rhodamine B, and various reactive dyes with decolorization rates between 10.9% and 61.9% in liquid Kirk medium [11]. Meanwhile, de Almeida et al. (2021) reported that *P. chrysosporium* achieved decolorization efficiencies of 82%, 89%, and 94% for direct yellow 27, reactive black 5, and reactive red 120, respectively, within 10 days [15]. Interestingly, the decolorization rates observed in the present study surpass those reported by Nihayah et al. (2018) [11], likely due to the use of the SSF approach. Unlike submerged fermentation, SSF more closely mimics the natural growth conditions of filamentous fungi and enhances the production of ligninolytic enzymes [16]. Sawdust as a lignocellulosic supporting material not only supports fungal colonization but also induces higher enzyme expression due to its structural complexity and limited nutrient availability, thereby improving overall decolorization performance.

### 3.4 Absorption Spectrum Profile in the Visible Beam Region

Changes in the UV–Visible (UV–Vis) absorption spectrum of Sujo batik wastewater before and after the decolorization treatment by *M. thermophila* and *P. chrysosporium* are presented in Figure 5. The initial spectrum, measured on day 0, exhibited a major absorption peak at $\lambda$max 540 nm, corresponding to the presence of chromophoric compounds with conjugated aromatic structures, which are characteristic of synthetic dyes used in batik production. After 14 days of treatment, a significant reduction in absorbance at this wavelength was observed in both fungal treatments, indicating substantial degradation of chromophores. Notably, *M. thermophila* showed a sharper decline in absorbance intensity compared to *P. chrysosporium*, which corresponds with the higher decolorization percentage recorded in previous analyses.

In addition to absorbance reduction, a slight hypsochromic shift (shift to shorter wavelength) in the absorption peak was also observed, suggesting structural modification or cleavage of conjugated aromatic systems in the dye molecules. These spectral changes confirm that the decolorization process was not merely due to adsorption but involved enzymatic breakdown of chromophoric structures by ligninolytic enzymes secreted during fungal metabolism. The near disappearance of the peak at 540 nm further supports the effective mineralization of dye molecules via the mycoremediation process. This is consistent with previous studies, which showed that lignin-degrading fungi are capable of disrupting aromatic dye structures, leading to color removal and detoxification of textile effluents [17].

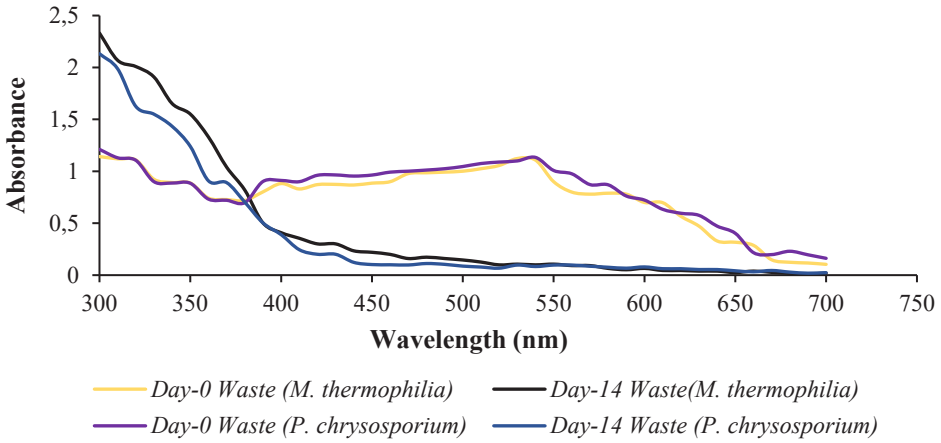


**Fig. 5.** Uv-Vis Spectra Before and After Decolorization by *M. thermophila* and *P. chrysosporium*

### 3.5 Ligninase Enzyme Activity

The production of ligninolytic enzymes by *M. thermophila* and *P. chrysosporium* was evaluated to understand their contribution to the decolorization of Sujo batik wastewater. As illustrated in Figure 6, both fungal isolates produced three key lignin-modifying enzymes (LMEs): lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), with varying activity levels throughout the 28-day incubation period. On day 0, *M. thermophila* exhibited initial laccase activity (2.98 U/mL), likely derived from residual media components or early metabolic responses. By day 7, LiP activity significantly increased to 15.32 U/mL, while laccase activity rose to 5.93 U/mL. The peak enzyme production occurred on day 14, with LiP, MnP, and laccase activities reaching 10.22 U/mL, 13.33 U/mL, and 8.91 U/mL, respectively. However, enzyme activity declined on days 21 and 28, indicating reduced metabolic activity or enzyme repression due to nutrient depletion. In comparison, *P. chrysosporium* showed detectable LiP, MnP, and laccase activities from day 7, with values of 4.30, 0.90, and 4.75 U/mL, respectively. These increased markedly by day 14 to 17.21 U/mL (LiP), 2.27 U/mL (MnP), and 7.12 U/mL (laccase). Similar to *M. thermophila*, enzyme activity declined thereafter, and by day 28, only MnP was marginally detectable (0.45 U/mL). The enzymatic activity profiles in this study were significantly higher than those reported in previous studies. Arinta et al. (2017) reported maximum LiP and MnP activities of 4.30 U/mL and 1.65 U/mL, respectively, by *M. thermophila* cultured in Kirk's medium with glucose as the carbon source [10]. Similarly, Susanti et al. (2016) reported LiP activity of only 0.86 U/mL for *P. chrysosporium* under similar conditions [18]. The increased enzyme productivity observed in this study is attributed to the solid-state fermentation (SSF) method using teak sawdust, which mimics the natural habitat of rot fungi and promotes higher ligninase expression.

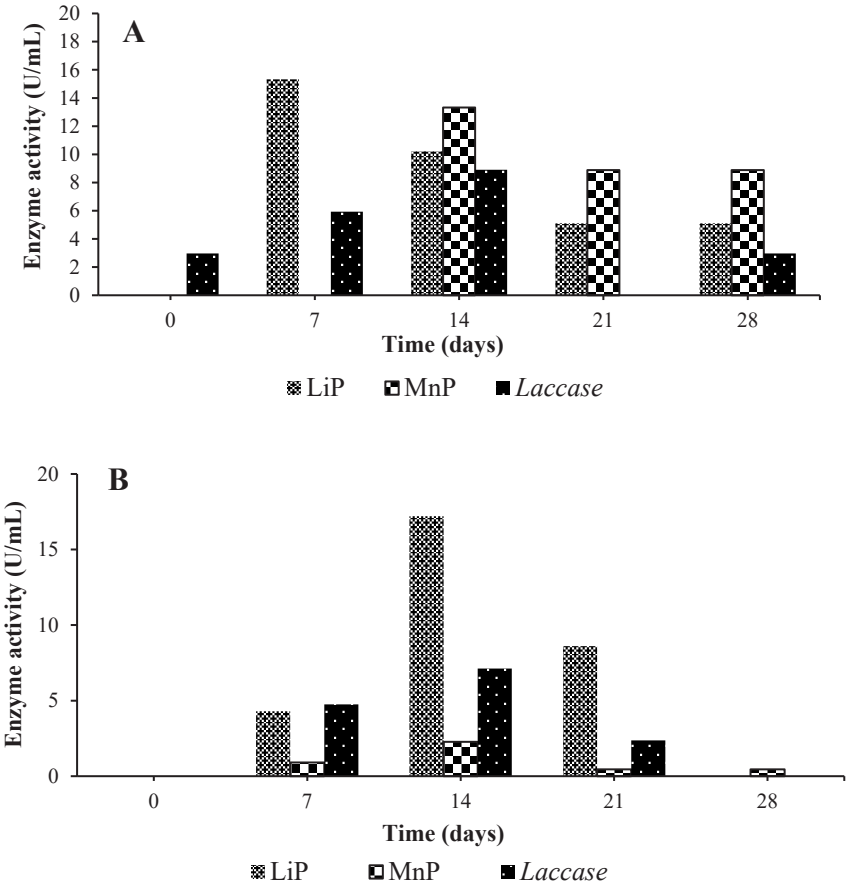


**Fig. 6.** Activity of Ligninase Enzyme: (A) *M. thermophila* isolate, (B) *P. chrysosporium* isolate

Teak sawdust provides a complex carbon source, rich in lignocellulosic material, which necessitates the secretion of extracellular oxidative enzymes by fungi to access usable sugars. This metabolic demand likely triggers overexpression of LMEs, particularly LiP and MnP. The strong correlation between enzyme activity and decolorization percentage (peak at day 14) further confirms the functional role of LMEs in dye degradation. Of particular interest, *M. thermophila* produced higher MnP activity relative to other enzymes on day 14, suggesting a primary role of MnP in the initial oxidation of phenolic structures. MnP oxidizes Mn(II) to Mn(III), which then forms stable complexes with organic acids (e.g., malonate, oxalate) secreted by the fungus. These Mn(III)-chelates act as diffusible redox mediators capable of oxidizing a wide range of phenolic and non-phenolic compounds. In contrast, *P. chrysosporium* exhibited dominant LiP activity during peak decolorization. This aligns with the known ligninolytic system of *P. chrysosporium*, which preferentially expresses LiP under nutrient-limited conditions. LiP catalyzes the oxidative cleavage of non-phenolic aromatic structures, facilitating breakdown of chromophoric groups and enhancing color removal. Taken together, the results suggest that *M. thermophila* holds potential as a robust MnP and LiP producer, while *P. chrysosporium* demonstrates greater LiP dominance. The relatively low and unstable production of laccase by both isolates is consistent with previous findings and suggests a secondary role of this enzyme in the decolorization of Sujo batik wastewater.

### 3.6 Characteristics of Sujo Batik Wastewater after Mycoremediation Treatment

The post-treatment quality of Sujo batik wastewater was evaluated based on changes in key physicochemical parameters pH, BOD, COD, TSS, total Cr and lethal concentration ($LC_{50}$), as shown in Table 4. The comparison includes untreated wastewater, wastewater treated through mycoremediation using *M. thermophila* and *P. chrysosporium*, and the regulatory thresholds specified by the Indonesian Ministry of Environment and Forestry Regulation No. P.16/MENLHK/SETJEN/KUM.1/4/2019.

**Table 4.** Parameters of Sujo Batik Wastewater after the Processing Process

| Parameters | Maximum Level (Quality Standart) | Sujo Batik Wastewater | Sujo Batik Wastewater after Mycomediation Treatment |
|---|---|---|---|
| pH | 6-9 | 11,58 | 6,82 |
| BOD (mg/L) | 60 | 3140 | 2036 |
| COD (mg/L) | 150 | 11600 | 6350 |
| TSS (mg/L) | 50 | 3376 | 87,2 |
| Cr total (mg/L) | 1,0 | 0,7110 | <0,0168 |
| $LC_{50}$ (ml/L) | - | 28,91 | 49,935 |

In the mycoremediation process, sawdust is used as a solid substrate during the SSF process. Sawdust is an organic material that will be used by the WRF as a nutrient. In order for the WRF to be able to use organic matter as nutrients, the WRF must degrade the organic matter, so that the value of BOD and COD increases. In addition, Sujo batik wastewater not only contains dyes and chemicals, but also contains fats and oils. During the mycoremediation process, the WRF will decolorize dyes and degrade organic materials in batik wastewater such as grease and oil. The more organic matter degraded by the WRF in batik wastewater, it will cause an increase in BOD and COD levels.

The $LC_{50}$ level of Sujo batik wastewater with an assumed concentration of 1000 ppm is 28.91 ml/L. The mycoremediation process succeeded in increasing the $LC_{50}$ of Sujo batik wastewater to 49,935 ml/L. Based on the $LC_{50}$ level obtained, it can be stated that the mycoremediation process is able to reduce the toxicity of Sujo batik wastewater. Based on the results of testing the parameters of Sujo batik wastewater after the processing process, it shows that the mycoremediation process is quite efficient and effective for processing Sujo batik wastewater before it is discharged into the environment because the total pH and Cr levels are in accordance with the set quality standards. The results of the mycomediation process produce a residue, in the form of sawdust with a mixture of waste and mold mycelium. The residue can be applied to the environment as a medium for planting ornamental plants.

The data demonstrate that mycoremediation was effective in significantly reducing all tested parameters. The most notable improvements were observed in pH, TSS, and total Cr, all of which met the established quality standards. Post-treatment pH was neutralized to 6.82, indicating efficient buffering and detoxification activity by the fungi. The total Cr level was reduced below detection limits (<0.0168 mg/L), a critical achievement given the potential carcinogenicity of hexavalent chromium compounds often present in textile dyes. Although BOD and COD values remained above the regulatory limits, both parameters showed substantial reductions (35.1% for BOD and 45.3% for COD), indicating partial biodegradation of organic pollutants. The persistence of elevated BOD and COD values may reflect the presence of recalcitrant organic compounds that require longer treatment duration or sequential remediation approaches. The $LC_{50}$ value increased from 28.91 mL/L in untreated wastewater to 49.935 mL/L after mycoremediation, suggesting a significant

reduction in acute toxicity. This improvement reflects the ability of white rot fungi to degrade not only chromophoric groups but also toxic aromatic compounds that contribute to wastewater lethality.

Sawdust, used as a supporting material in the SSF system, serves dual roles: providing a lignocellulosic carbon source and supporting fungal colonization. During mycoremediation, the fungi utilize the complex organic matter (e.g., cellulose, hemicellulose, lignin) in sawdust and break down toxicants in the wastewater. This enzymatic degradation process likely contributes to the observed reduction in toxicity and pollutant load.Moreover, the post-treatment residual biomass—a mixture of fungal mycelia, degraded sawdust, and adsorbed contaminants can potentially be repurposed as a soil amendment for ornamental plant cultivation. This aligns with principles of circular bioeconomy and valorization of bioremediation by-products. In summary, the mycoremediation process utilizing indigenous *M. thermophila* and *P. chrysosporium* in a solid-state fermentation system demonstrates promise as an environmentally sustainable and biologically efficient strategy for pre-treatment of batik wastewater prior to environmental discharge.

### 3.7 Study Limitations and Implications

This study demonstrated the potential of *M. thermophila* and *P. chrysosporium* in the mycoremediation of Sujo batik wastewater under controlled solid-state fermentation (SSF) conditions using sawdust as a substrate. However, several limitations should be acknowledged. First, the physicochemical characteristics of the wastewater may vary depending on the specific production batches, and these fluctuations were not assessed across multiple sampling periods. Second, the identification and quantification of specific dye compounds and aromatic intermediates were not performed, which could provide deeper insight into the enzymatic degradation pathways. Third, enzyme stability and gene expression related to ligninase production were not investigated, limiting our understanding of the regulatory mechanisms.

Despite these limitations, the study provides a promising outlook for the development of low-cost, eco-friendly, and sustainable bioremediation strategies using indigenous white rot fungi. The application of solid-state fermentation mimicking the fungi's natural habitat has been shown to enhance ligninolytic enzyme activity, supporting the scale-up of this method for industrial wastewater treatment. Further research is recommended to investigate the kinetics of enzyme production, degradation products, and the potential reuse of the final biomass as biofertilizer or soil amendment material.

## 4. Conclusions

This study demonstrated that the indigenous wood rot fungus *M. thermophila* possesses superior adaptability and bioremediation capacity in Sujo batik wastewater compared to *P. chrysosporium*. *M. thermophila* exhibited robust growth across all tested concentrations of wastewater and achieved the highest decolorization efficiency of 73.6% on day 14. In contrast, *P. chrysosporium* showed limited growth at higher concentrations and a lower decolorization efficiency of 63.8%. The superior performance of *M. thermophila* is closely linked to its ability to secrete higher levels of lignin-degrading enzymes, particularly manganese peroxidase (MnP) and lignin peroxidase (LiP), under solid-state fermentation conditions. Moreover, mycoremediation using *M. thermophila* significantly reduced the pH and total Cr content of the wastewater to within regulatory thresholds, while also decreasing its toxicity, as evidenced by $LC_{50}$ values. These findings suggest that *M. thermophila* is a promising candidate for the sustainable treatment of alkaline and heavily polluted textile effluents, especially those containing recalcitrant aromatic dyes.

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## REFERENCE

1. F. Mutiara, D. Hernawati, and V. Meylani, Differentiated of Batik Dyes and Environmental Effect in The Centre of Batik in Tasikmalaya City and Regency. Jurnal Presipitasi : Media Komunikasi dan Pengembangan Teknik Lingkungan. **19**, 3 (2022).
2. B. T. Pelosi, L. K. S. Lima, and M. G. A. Vieira, Removal of the synthetic dye Remazol Brilliant Blue R from textile industry wastewaters by biosorption on the Macrophyte *Salvinia natans*. Braz. J. Chem. Eng. **31.** 1035–1045 (2014).
3. M. Božič and V. Kokol, Ecological alternatives to the reduction and oxidation processes in dyeing with vat and sulphur dyes. Dyes and Pigments. **76**, 2, (2008).
4. E. B. Agustina and A. H. P. Yuniarto, Study of BOD, COD and TSS Removal in Batik Industry Wastewater using Electrocoagulation Method. JKPK (Jurnal Kimia dan Pendidikan Kimia). **7**, 2, (2022).
5. R. Jebapriya and J. Gnanadoss Joel, Bioremediation of textile dye using white rot fungi: A Review. International Journal of Current Research and Reviews. **05**, (2013).
6. M. Imran, D. Crowley, A. Khalid, S. Hussain, M. Mumtaz, and M. Arshad, Microbial biotechnology for decolorization of textile wastewaters. *R*eviews in Environmental Science and Bio/Technology. 14 (2014).
7. D. Sudrajat, N. Mulyana, and R. Heryani, Identifikasi *Phanerochaete chrysosporium* Yang Diiradiasi Sinar Gamma dengan Marka RAPD. Jurnal Ilmiah Aplikasi Isotop dan Radiasi. **14**, 1 (2018).
8. L. Delila, E. Susanti, and E. Sanjaya, Isolation and Screening of Indigenous Fungus Producing Lignin Peroxidase from the Cocoa Plantation in Sepawon Kediri Regency Indonesia. KnE Life Sciences. **3**, 131 (2017).
9. E. Susanti, T. Ardyati, Suharjono, and Aulani'am, Optimizing of lignin peroxidase production by the suspected novel strain of *Phanerochaete chrysosporium* ITB isolate, International Journal of ChemTech Research. **9**, 24–33 (2016).
10. A. Arinta, Penentuan jenis ligninase kapang pelapuk kayu indigenous isolat KLUM1, KLUM2, dan PnUM, Degree Thesis, Universitas Negeri Malang, Faculty of Mathematics and Natural Sciences (2017).
11. U. Nihayah, Skrining pewarna sintetis dan pengaruh aerasi terhadap dekolorisasi pewarna oleh kapang pelapuk kayu isolat KLUM1, dan KLUM2, Degree Thesis, Universitas Negeri Malang, Faculty of Mathematics and Natural Sciences (2018)
12. Permen LHK No. P.16/MENLHK/SETJEN/KUM.1/4/2019." Accessed: Jan. 29, 2023. [Online]. Available: https://jdih.maritim.go.id/en/peraturan-menteri-lingkungan-hidup-dan-kehutanan-no-p16menlhksetjenkum142019-tahun-2019
13. Susanti, E., Febriani, R. Apriani, D., Suharti, Alvionita, M. 2025. Decolorization of Reactive Black 5 by *Myceliophthora thermophila* KLUM1. 11 (1). 2025)
14. C. Lacina, G. Gourène, and S. Agathos, Utilization of Fungi for Bio-treatment of Raw Wastewaters. African Journal of Biotechnology. **2***,* 12 (2003).

15. A. P. de Almeida, A. Macrae, B. D. Ribeiro, and R. P. do Nascimento, Decolorization and detoxification of different azo dyes by *Phanerochaete chrysosporium* ME-446 under submerged fermentation. Braz J Microbiol. **52**, 2 (2021).
16. F. Boran and O. Yesilada, Enhanced production of laccase by fungi under solid substrate fermentation condition. BioResources. **6**, (2011).
17. R. Altohamy, J. Sun, M. Fareed, E.-R. Kenawy, and S. Ali, Eco Friendly biodegradation of Reactive Black 5 by newly isolated *Sterigmatomyces halophilus* SSA1575, valued for textile azo dye wastewater processing and detoxification. Scientific Reports. **10**, 12370 (2020).
18. E. Susanti, T. Ardyati, Suharjono, and Aulani'am, Optimizing lignin peroxidase production by the suspected novel strain of *Phanerochaete chrysosporium* ITB isolate. International Journal of ChemTech Research. **9**, 24–33 (2016).