Jurnal Biodjati 11(1): 90–103, May 2026

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Screening of Indigenous *Trichoderma* sp. for Remazol Red Decolorization and Phytotoxicity Assessment

Juanita Hibatullah*[1] and Rina Sri Kasiamdari[2]

Received: January 5, 2026
Revise from: January 16, 2026
Accepted: February 25, 2026

DOI: 10.15575/biodjati.v11i1.54096

[1]*Biology Program, Faculty of Science and Technology, Universitas Merangin, Jl. Jend Sudirman KM 4, Merangin, 37314 Indonesia.*

[2]*Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Yogyakarta, 55281, Indonesia.*

Corresponding author
**juanitahibatullah13@gmail.com*

Abstract. *The expansion of the batik industry has increased wastewater production, posing significant environmental challenges due to its complex organic composition and potential toxicity. Environmentally sustainable treatment strategies are required to mitigate the adverse impacts of batik effluents. This study aimed to isolate and evaluate indigenous fungal strains from batik wastewater environments in Surakarta for their ability to decolorize Remazol Red through enzymatic and biological processes. Fungal isolates were obtained from contaminated soil, sludge, and wastewater samples and screened for ligninolytic potential, followed by morphological characterization and enzyme activity assessment. The most effective isolate was further evaluated for decolorization efficiency under different dye concentrations and incubation periods, with concurrent analysis of fungal growth and changes in medium conditions during treatment. The environmental safety of the treated effluent was assessed using a plant-based phytotoxicity bioassay. The results indicated that Trichoderma sp. 1 exhibited the highest enzymatic performance, resulting in effective dye decolorization and reduced phytotoxicity compared to untreated effluent. The treated wastewater supported normal seed germination and early plant development, confirming successful detoxification. These findings suggest that locally sourced fungi represent a cost-effective and environmentally compatible solution for batik dye wastewater treatment and provide a scientific basis for developing fungal-based bioremediation strategies for textile effluent management.*

Keywords: *bioremediation, decolorization, detoxification, laccase, Trichoderma*

INTRODUCTION

Surakarta is one of the largest batik-producing cities in Indonesia, driving rapid growth in the industry and contributing to local economic development. However, many household-scale batik businesses and small- to medium-sized enterprises lack adequate waste treatment facilities. Disposal of waste without proper treatment that contains high levels of organic compounds poses a serious environmental risk, as these compounds can be carcinogenic in aquatic ecosystems by inhibiting sunlight penetration and oxygenation of water (Gokulan et al., 2019). Dye waste from batik production increases Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), negatively impacting river water quality due to ineffective dye waste management (Dewi et al., 2018).

Citation
Hibatullah, J. & Kasiamdari, R. S. (2026). Screening of Indigenous *Trichoderma* sp. for Remazol Red Decolorization and Phytotoxicity Assessment. *Jurnal Biodjati, 11*(1), 90-103.

Various physical, chemical, and biological methods have been reported to reduce the effects of dye waste. However, color removal treatments using chemical and physical methods have the disadvantage of requiring a large area and being expensive to manage. In addition, physical and chemical waste treatment generates byproducts, such as sludge, that are difficult to handle. The treatment of dye waste using biological agents is preferred because it requires relatively low maintenance costs. Biological agents can utilize existing carbon sources and adapt to polluted environments using microorganisms isolated from contaminated environments (Dewi, 2019). Fungi are potential candidates for the decolorization of azo dyes. They can adsorb dye molecules onto fungal mycelium via electrostatic interactions, followed by enzymatic breakdown of complex chemical bonds, thereby reducing color (Munir et al., 2018). Mohamed et al. (2019) successfully isolated and characterized *Aspergillus niger* capable of remediating dyes from Remazol Red waste. Safitri et al. (2020) also reported another fungal species, *Trichoderma viride*, which adsorbed 76.27% of the dye. Rohmawati & Kasiamdari (2022) stated that the fungus *T. yunnanense* successfully decolorized Indigosol Golden Yellow at a concentration of 250 ppm with an incubation time of 164 hours and a decolorization percentage of 93%.

Dye oxidation can occur in the presence of laccase, which catalyzes electron transfer to molecular oxygen. During this process, the dye undergoes electron loss, with electrons being relayed among Cu^{2+} ions within the enzyme's catalytic center, ultimately resulting in the four-electron reduction of oxygen to water (Morsy et al., 2020). Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) catalyzes the oxidation of a wide range of substrates, including methoxylated phenols, aromatic amines, phenolic acids, and several other compounds (Shanmugam et al., 2018).

Recent studies demonstrate that the effectiveness of biological treatment for azo dyes depends on microbial enzymatic activity, functional adaptation, and environmental conditions influencing dye decolorization. Genome-level analyses show that certain fungi decolorize dyes at 200 mg/L through oxidative mechanisms involving peroxidase-related enzymes, thereby significantly reducing aromatic amine intermediates and supporting fungal adaptation in dye-contaminated environments (Bulacio Gil et al., 2018). Metagenomic investigations indicate that functional cooperation among diverse microbial taxa enables decolorization efficiencies often exceeding 90% under alkaline and saline conditions (Guo et al., 2020). Optimized bacterial consortia can utilize reactive dyes as carbon sources, and post-treatment toxicity assessments in *Vigna radiata* demonstrate improved plant growth and reduced phytotoxicity after decolorization (Manogaran et al., 2021). Integrated multi-omics approaches highlight the importance of functional complementation within microbial communities, which enhances aromatic compound degradation and contributes to overall system stability in dye bioremediation (Balamurugan et al., 2025). These findings collectively support the implementation of integrated evaluation strategies that combine enzymatic performance, functional potential, and toxicity assessment to advance sustainable textile wastewater treatment technologies.

Numerous studies have investigated fungal dye treatment. However, most research lacks integrated screening approaches that combine enzymatic analysis, high-concentration dye decolorization efficiency, and environmental safety testing, particularly for indigenous fungi from Surakarta. This research addresses these limitations by isolating and identifying potential fungal isolates from local batik wastewater and employing a comprehensive methodology that assesses ligninolytic ability, laccase enzyme activity, decolorization capacity at various dye concentrations (500, 1000, 1500 ppm) with reduced incubation periods, and toxicity evaluation of the treated wastewater. This study hypothesizes that indigenous fungal isolates from batik wastewater environments exhibit effective laccase-mediated decolorization at high dye concentrations, thereby reducing phytotoxicity during seed germination. The results provide valuable insights for developing practical, cost-effective bioremediation technologies for textile effluent management.

MATERIALS AND METHODS

Study Site and Period

This study was initially conducted at Universitas Gadjah Mada in 2021 and subsequently continued as an extended research project from September 2024 to January 2025 at Universitas Merangin.

Sample Collection

Liquid waste samples were collected at the disposal site or waste storage area, with 100 mL of each sample collected and stored in a refrigerator at 4 °C. Soil and sludge samples were collected from the waste disposal site. Five grams of soil were collected from 15 cm below the surface using a shovel. The soil samples were placed in sterile, sealed containers and then filtered. After filtering, the soil samples were transferred to sterile bottles. All samples were collected in triplicate (n = 3). The soil and sludge samples were placed in 50 mL sterile polypropylene tubes and stored in Styrofoam containers with ice gel.

Fungi Isolation and Morphological Identification

Isolation was performed using the serial dilution method. The sample was mixed with 1 mL of sterile distilled water in a test tube and diluted to 10^{-4}. A 500 µL aliquot from the serial dilution was spread onto the surface of PDA medium supplemented with streptomycin at a concentration of 100 mg/L as a bacterial growth inhibitor. Purification was carried out by taking the mycelium from the edge of the medium aseptically and transferring it to fresh PDA medium, which was incubated at room temperature for seven days. Pure cultures on slant agar media were used as stock cultures and stored at 4 °C in a refrigerator.

Fungal colony morphology was characterized by cultivating pure isolates on PDA to assess macroscopic features, including colony surface texture, radial striations, concentric ring formation, growth rate, surface pigmentation, reverse coloration, and margin structure. Microscopic examination was conducted using the slide culture method, with samples stained with Lactophenol Cotton Blue to observe fungal microstructures. Identification was conducted at the genus level based on morphological characteristics in accordance with standard taxonomic keys for *Trichoderma*. Molecular identification using ITS sequencing was not performed.

Screening of Ligninolytic Activity of Fungal Isolates

Ligninolytic activity of the fungal isolates was screened using PDA medium supplemented with tannic acid. One plug of the fungal isolate was inoculated onto PDA medium containing 1% tannic acid and the antibacterial agent streptomycin, and the culture was incubated for 7 days (Dewi et al., 2018). Color changes were observed on the culture plates (Senthivelan et al., 2019). Each enzymatic assay was conducted in triplicate (n = 3). Positive results showed a brown zone on the selective medium, which was qualitatively assessed.

Laccase Activity

Laccase activity was quantified using a crude enzyme extract obtained after cultivation in a defined production medium. The medium comprised 5.0 g/L glucose, 5.0 g/L peptone, 1.0 g/L KH2PO4, 1.0 g/L ammonium acetate, 0.01 g/L MgSO4, 0.01 g/L CaCl2, 0.001 g/L MnSO4, 0.001 g/L $FeSO_4 \cdot 7H_2O$ and 0.0005 g/L CuSO4, adjusted to pH 6.0. Fungal inoculum was prepared by transferring two 5 mm mycelial plugs from seven-day-old cultures grown on PDA into 100 mL of the medium in 500 mL Erlenmeyer flasks. Cultures were incubated at 25 ± 3 °C with shaking at 150 rpm to facilitate enzyme production. All experiments were performed in triplicate (n = 3).

Jurnal Biodjati 11(1): 90–103 May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Laccase activity was assessed spectrophotometrically by monitoring the oxidation of 2.5 mM ABTS. The assay reaction mixture consisted of 1 mL of 2.5 mM ABTS, 1 mL of 0.1 M sodium acetate buffer at pH 4.5, and 1 mL of the crude enzyme extract. The rate of ABTS oxidation was determined by measuring the absorbance increase at 420 nm over 10 minutes. Enzyme activity units were defined as the amount of enzyme required to catalyze the oxidation of 1 µmol of ABTS per minute under the assay conditions (Patel & Bhaskaran, 2016). A blank control, substituting the enzyme extract with distilled water, was measured to correct for non-enzymatic oxidation. Laccase activity was determined using standard spectrophotometric principles, with careful consideration of reaction and enzyme volumes, optical path length, extinction coefficient, and reaction time, as recommended by Baltierra-Trejo et al., (2015). Laccase activity was calculated according to the following formula:

$$Laccase\ \ activity\ \ U/ml\ \ =\ \ \frac{\Delta A.min^{-1}\ \times\ V}{v\ \times\ \varepsilon\ \times\ d}$$

Where:
ΔA/min = Absorbance change per minute measured at 420 nm.
V = Total reaction volume in the assay mixture (mL).
V = Volume of crude enzyme extract used in the reaction (mL).
ε = Extinction coefficient of ABTS at 420 nm (36.000 M^{-1} cm^{-1})
d = optical path length of the cuvette (cm).

Decolorization Assay of Remazol Red Dye at Various Concentrations

The decolorization test was performed in Potato Dextrose Broth (PDB) medium supplemented with the dye. The decolorization test was performed by inoculating five plugs into 100 mL of 24 g/L PDB supplemented with Remazol Red dye at concentrations of 500, 1000, and 1500 ppm (Al-Tohamy et al., 2020 & Salem et al., 2019). Erlenmeyer flasks containing fungi and dye were then shaken at 150 rpm for 120 hours at room temperature (Abd El-Rahim et al., 2017). All treatments were conducted in triplicate (n = 3). The percentage of decolorization of the supernatant was measured using a UV-Vis spectrophotometer. The absorbance of the samples before and after treatment was measured at a wavelength (λ) of 530 nm for Remazol Red (Mohamed et al., 2019). The percentage of decolorization was measured using the following formula:

$$Percentage\ \ \ decolorization\ \ =\ \ \frac{Initial\ \ \ Absorbation\ \ -\ \ \ Final\ \ \ Absorbation}{Initial\ \ \ Absorbation}\ \ \times\ \ 100\%$$

Fungal Biomass

Fungal biomass was measured using 30 mL of PDB supplemented with various concentrations of dye (500, 1000, and 1500 ppm) and inoculated with one fungal plug under aseptic conditions. The culture was then shaken at 150 rpm. Biomass was determined at 24-hour intervals using filter paper. The samples were then dried at 105 °C and weighed until constant weight was achieved. Biomass determination was performed in triplicate (n = 3) (Hadibarata et al., 2018).

Phytotoxicity Test of the most potent fungi isolate

Decolorization products derived from the most effective isolates and from water (control) were prepared for the phytotoxicity test. Twenty mung bean seeds (*Vigna radiata* L) were planted in sterilized soil. The experiment involved irrigating each seedling with 5 mL of the respective treatment. After five days, seed germination percentage, shoot (plumule) length, and root (radicle) length were measured to assess plant growth (Gao et al., 2020). Each treatment was conducted in triplicate (n = 3).

Data Analysis

All data were presented as mean ± standard deviation (SD) based on three independent replicates. Statistical analyses were performed using SPSS version 25.0. Two-way analysis of variance (ANOVA) was used to evaluate the effects of incubation time and dye concentration on decolorization efficiency. Duncan's multiple-range test was applied to determine significant differences at the 0.001 significance level. Subsequently, the most effective isolate was selected for detailed analysis and discussion.

RESULTS AND DISCUSSION

Isolation and Screening Ligninolytic Activity, and Morphological Identification of Fungal Isolates

Samples were collected from four home industries in Surakarta, yielding 12 samples: four soil, four sludge, and four water samples contaminated with batik wastewater from dyeing sites and waste channels. Qualitative screening for ligninolytic activity was indicated by the formation of brown zones surrounding fungal colonies, which were subsequently scored. Three isolates demonstrated significant ligninolytic ability and were labeled 24 SPX, 12 SPL, and 19 SPS. The brown zones were classified according to their ligninolytic capacity (Table 1). Isolates 12 SPL, 19 SPS, and 24 SPX showed the same ligninolytic activity. Although several isolates demonstrated similar ligninolytic screening results, subsequent quantitative laccase activity and decolorization assays were required to identify the most promising isolate. Illuri et al. (2021) stated that the brown halo surrounding colonies reflects total polyphenol oxidase activity. Ayu & Kasiamdari, (2022); Dewi et al. (2018); and Rohmawati & Kasiamdari, (2022), successfully isolated fungal strains with the potential to decolorize Remazol Black, Indigosol Blue, and Indigosol Yellow dyes. This finding suggests that tannic acid screening may serve as a preliminary indicator for selecting fungi with potential for dye decolorization. In contrast to Alfarra et al. (2013), tannic acid cannot be used as the sole test to confirm that the enzyme produced is laccase. Therefore, enzymatic activity and decolorization assays were further conducted to confirm the capability.

Table 1. Scoring of ligninolytic ability of three potential isolates

Isolates	Brown zone scoring			
	+	++	+++	++++
24 SPX			+++	
12 SPL			+++	
19 SPS			+++	

Morphological characterization of the fungal isolates revealed that they belonged to the same genus. Under microscopic observation (Table 2), isolates 24 SPX, 12 SPL, and 19 SPS shared similar characteristics, including septate hyphae, verticillate and branched conidiophores with two to three phialides, and round conidia, consistent with *Trichoderma* sp. Identification was conducted at the genus level using morphological characteristics. However, molecular confirmation via ITS sequencing provides greater taxonomic resolution and should be incorporated into future studies aimed at strain-level characterization.

Jurnal Biodjati 11(1): 90–103 May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Table 2. Morphological identification of three potential fungal isolates

Isolates Code	Surface Colony Color	Surface Texture	Microscopic Characteristics	Identification
24 SPX	Green	Granular	Conidiophores, conidia, and phialides	*Trichoderma* sp. 1
12 SPL	Light green	Cottony	Conidiophores, conidia, and phialides	*Trichoderma* sp. 2
19 SPS	Light green	Cottony	Conidiophores, conidia, and phialides	*Trichoderma* sp. 3

Laccase Activity

Laccase activity measurements indicated variation among fungal isolates during the 120-hour incubation period (Figure 1). Isolate *Trichoderma* sp. 1 showed the highest laccase enzyme activity compared to other potential isolates, with peak activity at 72 hours of incubation at 3.67 U/mL, followed by isolates *Trichoderma* sp. 2 with enzyme activities of 1.84 U/mL at 72 hours of incubation. Meanwhile, isolate *Trichoderma* sp. 3 showed the highest activity at 98 hours with 1.66 U/mL. The highest Laccase activity varied among fungal isolates, exhibiting distinct temporal expression profiles. The laccase activity of *Trichoderma* sp. 1 fell within a moderate but functionally relevant range when compared with well-established ligninolytic fungi. For instance, *Pleurotus ostreatus* HK35 produced crude laccase activity of approximately 3.48 U/mL when cultivated on rice bran medium (Isanapong et al., 2024). Although the absolute laccase activity of *Trichoderma* sp. 1 was lower than that of purified laccase preparations from Pleurotus species, its relatively rapid peak production within 72 hours demonstrated a significant advantage in terms of reduced induction time and accelerated enzymatic response. *Phanerochaete chrysosporium* has been reported to exhibit enhanced laccase production primarily under co-cultivation conditions, where synergistic interactions with other white-rot fungi significantly stimulate enzyme expression (Singh et al., 2020).

Comparable temporal patterns of laccase production have been reported in other *Trichoderma* species, although absolute enzyme activity may vary depending on strain characteristics and cultivation conditions. For example, Adnan et al. (2015) observed peak laccase activities of 5.8 U/mL in *T. atroviride* F03 after 98 hours of incubation. Variations in laccase activity among fungal species reflect differences in enzyme secretion pathways and the genetic diversity among isolates. Overall, these comparisons indicate that *Trichoderma* sp. 1 displayed competitive early-stage laccase production and effective functional activity. This balance between production rate and enzymatic efficiency supported the suitability of *Trichoderma* sp. 1 as a practical ligninolytic agent for dye decolorization processes under relatively short incubation periods. Several factors influenced enzyme activity variation, including the formulation of the cultivation medium, the endogenous laccase biosynthesis capacity of fungi, and growth environment parameters that control the level and timing of enzyme expression.

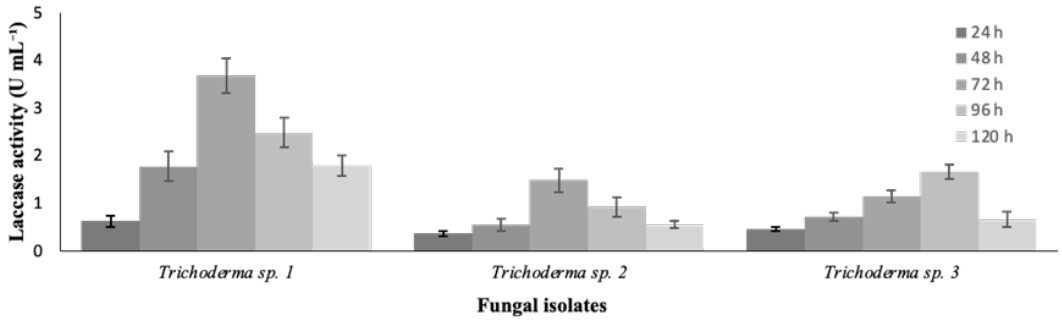

Figure 1. Time-course of laccase activity in three *Trichoderma* isolates during 120 h incubation. Error bars represent mean ± standard deviation (n = 3)

Decolorization of Remazol Red at Various Concentration

The highest laccase activity observed in *Trichoderma* sp. 1 likely contributed to its superior and faster decolorization performance, indicating a functional linkage between enzymatic production and dye removal kinetics. As shown in (Figure 2), isolate *Trichoderma* sp. 1 exhibited the highest decolorization activity across varying concentrations of Remazol Red dye, followed by *Trichoderma* sp. 2, and *Trichoderma* sp. 3. *Trichoderma* sp. 1 achieved a maximum decolorization of 72.51% at 500 ppm following 120 hours of incubation, while decolorization decreased to 48.96% and 32.89% at 1000 ppm and 1500 ppm, respectively. The visual progression of decolorization by *Trichoderma* sp. 1 at all concentrations and incubation times (Figure 3). The observed decolorization may involve adsorption of dye molecules onto fungal hyphae, followed by enzymatic degradation, as reported in previous studies. (Kaur et al., 2015; Zainip et al., 2021). However, the present study did not experimentally distinguish between adsorption and enzymatic degradation mechanisms. Based on Figure 2, the higher the dye concentration in the medium, the greater the inhibition of decolorization activity. According to Parmar (2014), dye concentration influences the decolorization activity of Acridine Acid dye. *Aspergillus* sp. decolorized efficiently at a concentration of 100 ppm, whereas higher dye concentrations resulted in decreased decolorization ability. Afiya et al. (2019) reported that dye decolorization at initial concentrations ranging from 10 to 125 mg/L decreased with increasing dye concentration. Maximum decolorization by *Coriolus versicolor* and *Pleurotus ostreatus* occurred at 10 mg/L, with 94%-83% decolorization, and decreased gradually at higher dye concentrations. Similarly, Safitri et al. (2020) reported that *T. viride* showed reduced decolorization of the Remazol Brilliant Violet dye at higher concentrations, which was associated with increased dye toxicity.

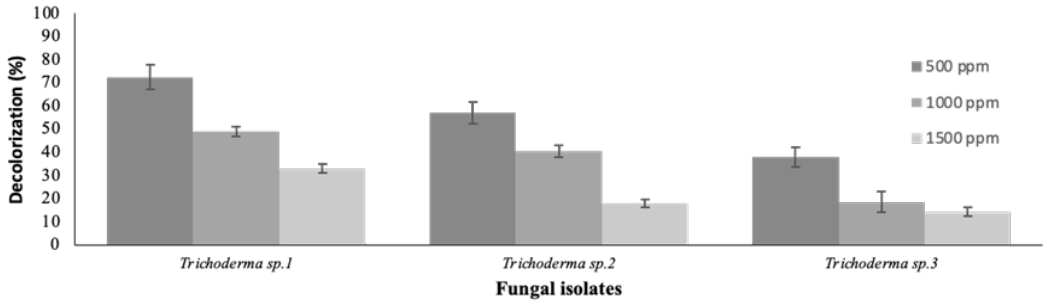

Figure 2. Decolorization efficiency of Remazol Red by three *Trichoderma* isolates at 500, 1000, and 1500 ppm after 120 h of incubation. Error bars represent mean ± standard deviation (n = 3).

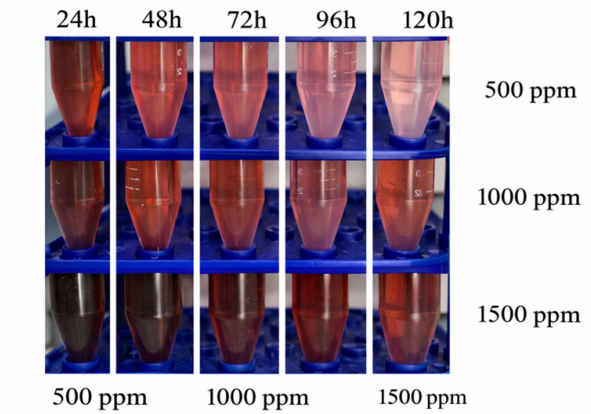

Figure 3. Visual progression of Remazol Red decolorization by *Trichoderma* sp. 1 at 500, 1000, and 1500 ppm over 24–120 h of incubation.

Jurnal Biodjati 11(1): 90–103 May 2026

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Growth Profile of Three Potential Isolates in Remazol Red at Various Concentrations

Measurements of fungal dry weight decreased gradually as the medium dye concentration increased, indicating an inhibitory effect on fungal growth at higher concentrations. Among the isolates, *Trichoderma* sp. 3 exhibited the greatest dry biomass when exposed to Remazol Red at 500, 1000, and 1500 ppm, with recorded weights of 0.27 g, 0.24 g, and 0.20 g, respectively (Figure 4). Conversely, isolate *Trichoderma* sp. 1 showed the lowest dry weight across all test concentrations: 0.22 g, 0.20 g, and 0.15 g. Fungal biomass decreased with increasing dye concentration in the medium. This indicates that high dye concentrations can inhibit fungal growth. Ranjusha et al. (2010) found that a decrease in the total biomass production of *Aspergillus flavus* at a dye concentration of 1000 mg/L caused the total biomass to decrease significantly to 1.44 g/L, indicating a change in the glucose concentration in the growth medium that had an initial dye concentration of 0-1000 mg/L. Media without dye allowed fungi to utilize glucose efficiently. Glucose consumption decreased with increasing initial dye concentration, indicating that higher concentrations inhibited fungal biomass (Mohamed et al., 2019).(Mohamed et al., 2019).

The absence of a direct correlation between fungal biomass and decolorization efficiency demonstrated that enzymatic activity, rather than fungal growth, primarily governs dye removal. Based on Figures 2 and 4, high fungal growth rates were not always directly proportional to decolorization activity. Isolate *Trichoderma* sp. 1 showed the highest decolorization activity (72.51%) at 500 ppm, with a dry weight of 0.22 g. In contrast, isolate *Trichoderma* sp. 3 had the highest dry weight (0.27 g) at 500 ppm Remazol Red dye, but only achieved 37.86% decolorization. These findings are in line with Dewi (2019), who reported that *Aspergillus* sp. 5 isolates had the highest dry weight of 0.370 g, whereas the decolorization activity obtained was only 21.04%.

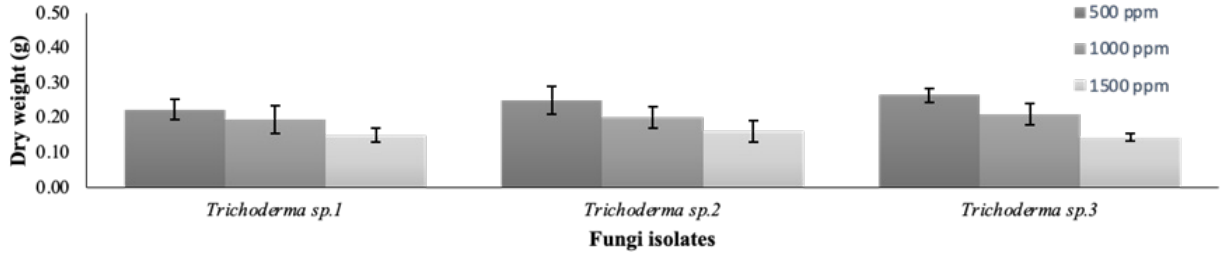

Figure 4. Dry biomass production of three *Trichoderma* isolates cultured in Remazol Red at 500, 1000, and 1500 ppm after 120 h of incubation. Error bars represent mean ± standard deviation (n = 3).

Evaluation of *Trichoderma* sp. 1 Decolorization Under Diverse Remazol Red Concentration, Incubation Time, and Associated pH Change

Based on the screening results, *Trichoderma* sp. 1 was selected for further statistical analysis due to its superior ligninolytic activity and decolorization performance. Two-way ANOVA revealed significant main effects of incubation time ($F(4, 30) = 3631.00$, $p < 0.001$) and dye concentration ($F(2, 30) = 17828.10$, $p < 0.001$) on Remazol Red decolorization efficiency. A significant interaction between incubation time and dye concentration was also observed ($F(8, 30) = 219.04$, $p < 0.001$). Post hoc analysis using Duncan's multiple range test confirmed significant differences among treatments, as indicated by different superscript letters (Table 3).

Decolorization efficiency increased with decreasing dye concentration and increasing incubation time. A dye concentration of 500 ppm yielded the highest decolorization efficiency, reaching 72.51% after 120 hours of incubation, which was significantly higher than that of other treatments. In contrast, at 1500 ppm, decolorization efficiency remained low (32.89–48.96%) across all incu-

Table 3. Effects of dye concentration and incubation time on Remazol Red decolorization and pH change during decolorization by *Trichoderma* sp. 1

Dye concentration (ppm)	Incubation (Hour)	Decolorization (%)	pH
500	24	38.84 ± 1.49^{d}	7.00 ± 0.02
	48	50.07 ± 1.85^{c}	7.40 ± 0.01
	72	64.76 ± 0.59^{b}	7.10 ± 0.01
	96	71.35 ± 1.04^{a}	6.88 ± 0.02
	120	72.51 ± 0.11^{a}	7.17 ± 0.05
1000	24	29.89 ± 0.41^{e}	7.50 ± 0.03
	48	31.09 ± 0.78^{e}	7.80 ± 0.24
	72	39.41 ± 0.71^{d}	7.44 ± 0.16
	96	47.41 ± 0.10^{c}	7.15 ± 0.07
	120	48.96 ± 1.13^{c}	7.39 ± 0.28
1500	24	15.74 ± 0.23^{g}	7.90 ± 0.30
	48	19.47 ± 0.59^{f}	8.23 ± 0.33
	72	22.93 ± 0.46^{f}	8.15 ± 0.15
	96	32.41 ± 0.17^{de}	8.20 ± 0.42
	120	32.89 ± 0.06^{de}	8.28 ± 0.66

Note: Values are presented as mean ± standard deviation. Different superscript letters indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.001$).

bation times, indicating inhibition of decolorization at higher dye concentrations. Changes in pH during the decolorization process ranged from 6.88 to 8.28, indicating that decolorization occurred under neutral to slightly alkaline conditions.

The decline in decolorization efficiency with increasing dye concentration indicates that Remazol Red inhibits the metabolic and enzymatic activity of *Trichoderma* sp. 1, particularly at higher concentrations. Increased dye levels disrupt fungal growth and ligninolytic enzyme systems by inducing oxidative stress or limiting substrate accessibility. This disruption explains the reduced decolorization observed at 1000 and 1500 ppm, even after prolonged incubation. Conversely, extended incubation periods generally enhance decolorization, indicating that sufficient contact time is required for enzymatic adaptation and dye degradation. However, once enzymatic activity stabilizes or substrate inhibition occurs, prolonged incubation may not further enhance dye removal. The consistently lower Duncan groupings at higher dye concentrations confirm that pollutant load, rather than incubation time, is the primary determinant of decolorization efficiency. These findings highlight the importance of optimizing both dye concentration and retention time in fungal-based bioremediation to balance enzymatic capacity and reduce toxic stress.

Incubation time significantly affected the decolorization efficiency of dyes by *Trichoderma* sp. 1 at all concentrations tested, with a gradual increase in decolorization efficiency observed as incubation time extended from 24 to 120 hours. At a concentration of 500 ppm, decolorization efficiency increased over time, rising from 38.84% at 24 hours to 72.51% at 120 hours. This pattern is consistent with enzymatic biodegradation kinetics, in which fungi require time to produce degradative laccase enzymes. These results align with Parshetti et al. (2007), who observed that the removal of Reactive Blue-25 by *A. ochraceus* NCIM-1146 required longer incubation as dye concentration increased. *A. ochraceus* NCIM-1146 achieved maximum decolorization after 20 days of incubation at a dye concentration of 100 mg/L.

Changes in medium pH during the decolorization process showed a pattern that correlated with dye concentration and biodegradation efficiency. At a concentration of 500 ppm, the medium pH remained relatively stable at 6.88–7.40, which represents optimal conditions for the activity of ligninolytic enzymes responsible for decolorization. Ning et al. (2018) and Nabeela et al. (2023) stated that pH is important in decolorization because it is closely related to degradative enzyme activity. Under alkaline conditions, enzyme activity can be reduced, resulting in more efficient color removal under neutral to slightly acidic conditions. The observed shift toward alkaline conditions at higher dye concentrations coincided with reduced decolorization efficiency, indicating decreased stability and activity of ligninolytic enzymes under alkaline conditions.

Phytotoxicity Test of Decolorization Product by *Trichoderma* sp. 1

A reduction in germination percentage and seedling growth of *Vigna radiata* exposed to untreated dye demonstrates the presence of toxic compounds that inhibit metabolic activity and cell division during germination. Higher dye concentrations resulted in pronounced growth inhibition, with germination rates declining from 55% to 20% as concentrations increased from 500 to 1500 ppm (Table 4). This trend suggests that the accumulation of dye substances in seed tissues disrupts essential physiological processes, such as water uptake, mobilization of food reserves, and biosynthesis of growth-regulating hormones. A substantial decrease in plumule length from 4.52 cm to 1.36 cm was observed with increasing dye concentrations. In contrast, radicle length showed only minor variation, ranging from 1.56 cm to 1.58 cm, suggesting that shoot development was more sensitive to dye-induced stress than root elongation. Comparable phytotoxic responses in *V. radiata* have been documented by Dewi et al. (2018), who observed that untreated dye effluents suppressed normal seedling development.

In contrast, the lack of phytotoxic effects in the decolorized effluent demonstrates a significant reduction in dye toxicity, as evidenced by normal seed germination and seedling development comparable to the control (Table 4). This finding suggests that chemical modification of dye molecules occurred during the decolorization process, involving the degradation of chromophore and auxochrome structures into simpler compounds. Chen et al. (2019) reported that decolorization of triphenylmethane dyes by *Bjerkandera adusta* SWUSI4 is accompanied by enzymatic transformation, during which ligninolytic enzymes cleave complex dye structures into simpler aromatic compounds with reduced biological toxicity.

The restoration of seed germination and seedling growth after decolorization indicates that reduced phytotoxicity results from chemical transformation of dye molecules rather than solely from color removal. The degradation of complex dye structures typically produces low-molecular-weight aromatic metabolites, such as aromatic amines and sulfonated aromatic compounds, which are less bioreactive than the original dyes. Metabolomic studies by Sun et al. (2017) showed that microbial metabolism of azo dyes lessened disruptions in key cellular metabolic pathways, particularly those related to energy metabolism, suggesting that these transformation products impose reduced biological stress. Similarly, Singh et al. (2024) found that biodegradation products of Reactive Green 12 supported normal plant growth and eliminated phytotoxic effects. Although complete mineralization was not evaluated, the observed reduction in phytotoxicity indicates that the metabolic byproducts generated during fungal decolorization are less environmentally harmful and pose a reduced risk to non-target plant species. However, it should be noted that metabolite profiling was not performed in this study. Therefore, the proposed detoxification mechanism is inferred from phytotoxicity outcomes and supported by previous literature rather than direct chemical identification of transformation products. Further studies are required to confirm the specific degradation pathway and metabolite composition.

Table 4. Effects of decolorization products on germination percentage, plumule length, and radicle length of *Vigna radiata* L.

Dye concentration (ppm)	Percentage of germination (%)	Plumule (cm)	Radicle (cm)
Control	100	12.50±0.65	6.00±1.20
500	55	4.52±0.47	1.56±0.05
1000	35	3.24±1.28	1.72±0.03
1500	20	1.36±0.04	1.58±0.06
Decolorized 500	100	11.20±0.84	5.80±0.20
Decolorized 1000	100	9.38±0.74	4.61±0.59
Decolorized 1500	100	8.68±0.55	3.83±0.39

Note : Values are presented as mean ± standard deviation.

CONCLUSION

Among the tested isolates, indigenous *Trichoderma* sp. 1 showed the greatest potential for bioremediation of Remazol Red, as evidenced by strong ligninolytic activity and elevated laccase production. This isolate efficiently removed dye color at moderate concentrations and incubation periods, underscoring its suitability for fungal-based dye treatment. Furthermore, phytotoxicity assessments confirmed that the decolorized effluent did not inhibit seed germination or early plant growth, demonstrating effective detoxification and enhanced environmental compatibility. Collectively, these results indicate that indigenous *Trichoderma* sp. represents an effective and environmentally safe biological agent for treating textile dye wastewater, especially in small-scale or cost-sensitive remediation systems.

AUTHOR CONTRIBUTION

J.H. and **R.S.K.** designed the study. **JH** conducted the experiments in the laboratory, analyzed data, and wrote the first draft of the manuscript. **R.S.K.** and **J.H**. revised and finalized the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Faculty of Biology Universitas Gadjah Mada and Biology program of Universitas of Merangin for providing the necessary research facilities and support for completing this work. This manuscript is part of the **J.H.** thesis under the supervision of **R.S.K.**

CONFLICT OF INTEREST

The authors declare there is no conflict of interest concerning the present paper

REFERENCES

Abd El-Rahim, W. M., Moawad, H., Abdel, A. A. Z., & Sadowsky, M. J. (2017). Optimization of Conditions for Decolorization of Azo-Based Textile Dyes by Multiple Fungal Species. *Journal of Biotechnology*. *260*: 11–17. DOI: https://doi.org/10.1016/j.jbiotec.2017.08.022

Adnan, L. A., Sathishkumar, P., Mohd, Y. A. R., & Hadibarata, T. (2015). Metabolites Characterisation of Laccase Mediated Reactive Black 5 Biodegradation by Fast Growing Ascomycete Fungus *Trichoderma atroviride* F03. *International Biodeterioration and Biodegradation*. *104*: 274–282. DOI: https://doi.org/10.1016/j.ibiod.2015.05.019

Afiya, H., Ahmet, E. E., & 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): 1–11. DOI: https://doi.org/10.21839/jaar.2019.v4i1.260

Alfarra, H. Y., Hasali, N. H. M., & Omar, M. N. (2013). A Lignolytic Fungi with Laccase Activity Isolated from Malaysian Local Environment for Phytochemical Transformation Purposes. *International Research Journal of Biological Sciences*. Retrieved from https://www.isca.me/IJBS/Archive/v2/i2/10.ISCA-IRJBS-2012-226.pdf

Al-Tohamy, R., Kenawy, E. R., Sun, J., & Ali, S. S. (2020). Performance of a Newly Isolated Salt-Tolerant Yeast Strain *Sterigmatomyces halophilus* SSA-1575 for Azo Dye Decolorization and Detoxification. *Frontiers in Microbiology*. *11*: 1–19. DOI: https://doi.org/10.3389/fmicb.2020.01163

Ayu, Y. S., & Kasiamdari, R. S. (2022). Screening and Identification of Fungi Isolated from Batik Wastewaters for Decolorization of Remazol Black B Dye and Batik Effluent. *Journal of Degraded and Mining Lands Management*. *10*(1): 3829–3839. DOI: https://doi.org/10.15243/jdmlm.2022.101.3829

Balamurugan, J., Jagadeesan, H., & Vijayakumar, M. (2025). A Metagenomic Approach to Predict the Role of Microbiome in a Plant-Microbe System for Degrading the Model Azo Dye Methyl Red. *Journal of Contaminant Hydrology*. *275*: 1–15. DOI: https://doi.org/10.1016/j.jconhyd.2025.104703

Baltierra-Trejo, E., Márquez-Benavides, L., & Sánchez-Yáñez, J. M. (2015). Inconsistencies and Ambiguities in Calculating Enzyme Activity: The Case of Laccase. *Journal of Microbiological Methods*. *119*: 126–131. DOI: https://doi.org/10.1016/j.mimet.2015.10.007

Bulacio Gil, N. M., Pajot, H. F., Rosales Soro, M. del M., de Figueroa, L. I. C., & Kurth, D. (2018). Genome-Wide Overview of *Trichosporon akiyoshidainum* HP-2023, New Insights into its Mechanism of Dye Discoloration. 3 *Biotech. 8*(10). DOI: https://doi.org/10.1007/s13205-018-1465-y

Chen, S. H., Cheow, Y. L., Ng, S. L., & Ting, A. S. Y. (2019). Biodegradation of Triphenylmethane Dyes by Non-white Rot Fungus *Penicillium simplicissimum*: Enzymatic and Toxicity Studies. *International Journal of Environmental Research. 13*(2): 273–282. DOI: https://doi.org/10.1007/s41742-019-00171-2

Dewi, R. S., Kasiamdari, R. S., Martani, E., & Purwestri, Y. A. (2018). Decolorization and Detoxification of Batik Dye Effluent Containing Indigosol Blue-04B Using Fungi Isolated from Contaminated Dye Effluent. *Indonesian Journal of Biotechnology. 23*(2): 54–60. DOI: https://doi.org/10.22146/ijbiotech.32332

Dewi, R. S., (2019). Fungi Limbah Industri Batik Sebagai Pewarna Batik Indigosol Blue O4b. Dissertation. Universitas Gadjah Mada, Indonesia

Gao, T., Qin, D., Zuo, S., Peng, Y., Xu, J., Yu, B., Song, H., & Dong, J. (2020). Decolorization and Detoxification of Triphenylmethane Dyes by Isolated Endophytic Fungus *Bjerkandera adusta* SWUSI4 Under Non-Nutritive Conditions. *Bioresources and Bioprocessing*. *7*(1): 1–12. DOI: https://doi.org/10.1186/s40643-020-00340-8

Gokulan, R., Avinash, A., Prabhu, G. G., & Jegan, J. (2019). Remediation of Remazol Dyes by Biochar Derived from *Caulerpa scalpelliformis* - An Eco-Friendly Approach. J*ournal of Environmental Chemical Engineering. 7*(5): 1–9. DOI: https://doi.org/10.1016/j.jece.2019.103297

Guo, G., Hao, J., Tian, F., Liu, C., Ding, K., Xu, J., Zhou, W., & Guan, Z. (2020). Decolorization and Detoxification of Azo Dye by Halo-Alkaliphilic Bacterial Consortium: Systematic Investigations of Performance, Pathway and Metagenome. *Ecotoxicology and Environmental Safety. 204*. DOI: https://doi.org/10.1016/j.ecoenv.2020.111073

Hadibarata, T., Syafiuddin, A., Al-Dhabaan, F. A., Elshikh, M. S., & Rubiyatno. (2018). Biodegradation of Mordant orange-1 Using Newly Isolated Strain *Trichoderma harzianum* RY44 and its Metabolite Appraisal. *Bioprocess and Biosystems Engineering. 41*(5): 621–632. DOI: https://doi.org/10.1007/s00449-018-1897-0

Illuri, R., Kumar, M., Eyini, M., Veeramanikandan, V., Almaary, K. S., Elbadawi, Y. B., Biraqdar, M. A., & Balaji, P. (2021). Production, Partial Purification and Characterization of Ligninolytic Enzymes from Selected Basidiomycetes Mushroom Fungi. S*audi Journal of Biological Sciences. 28*(12): 7207–7218. https://doi.org/10.1016/j.sjbs.2021.08.026

Isanapong, J., Suwannoi, K., Lertlattanapong, S., & Panchal, S. (2024). Purification, Characterization of Laccase from *Pleurotus ostreatus* HK35, and Optimization for Congo Red Biodecolorization Using Box–behnken Design. *3 Biotech. 14*(3). DOI: https://doi.org/10.1007/s13205-024-03926-7

Kaur, B., Kumar, B., Garg, N., & Kaur, N. (2015). Statistical Optimization of Conditions for Decolorization of Synthetic Dyes by *Cordyceps militaris* MTCC 3936 Using RSM. *BioMed Research International. 1*: 1–17. DOI: https://doi.org/10.1155/2015/536745

Manogaran, M., Yasid, N. A., Othman, A. R., Gunasekaran, B., Halmi, M. I. E., & Shukor, M. Y. A. (2021). Biodecolourisation of Reactive Red 120 as A Sole Carbon Source by A Bacterial Consortium—Toxicity Assessment and Statistical Optimisation. *International Journal of Environmental Research and Public Health. 18*(5): 1–26. DOI: https://doi.org/10.3390/ijerph18052424

Mohamed, A. M., Abduo, D. A. M., Karam Al- Dien, A. A., Ramadan, E. M., & Abd Elrazek, T. M. (2019). Decolorization of Remazol Blue and Remazol Red Using *Aspergillus niger* Isolated from Textile Wastewater. *Journal of Environmental Science. 45*(1): 1–18. DOI: https://doi.org/10.21608/jes.2019.36936

Morsy, S. A. G. Z., Ahmad Tajudin, A., Ali, M. S. M., & Shariff, F. M. (2020). Current Development in Decolorization of Synthetic Dyes by Immobilized Laccases. *Frontiers in Microbiology, 11*: 1–8. DOI: https://doi.org/10.3389/fmicb.2020.572309

Munir, E., Rahayu, V., Priyani, N., & Yurnaliza. (2018). Decolorization of Batik Naphthol Dye by Local Ligninolytic Fungal Isolates. *Journal of Physics: Conference Series. 1116*(5): 1–7. DOI: https://doi.org/10.1088/1742-6596/1116/5/052043

Nabeela, Khan, S. A., Mehmood, S., Shabbir, S. Bin, Ali, S., Alrefaei, A. F., Albeshr, M. F., & Hamayun, M. (2023). Efficacy of Fungi in the Decolorization and Detoxification of Remazol Brilliant Blue Dye in Aquatic Environments. *Microorganisms. 11*(3): 1–19. DOI: https://doi.org/10.3390/microorganisms11030703

Ning, C., Qingyun, L., Aixing, T., Wei, S., & Youyan, L. (2018). Decolorization of a Variety of Dyes by *Aspergillus flavus* A5p1. *Bioprocess and Biosystems Engineering. 41*(4): 511–518. DOI: https://doi.org/10.1007/s00449-017-1885-9

Parmar, P. R. (2014). Decolorization of Acridine Red Dye by The Fungi Aspergillus Species. *Journal of Scientific and Innovative Research*. Retrieved from http://www.jsirjournal.com/Vol3_Issue4_10.pdf

Parshetti, G. K., Kalme, S. D., Gomare, S. S., & Govindwar, S. P. (2007). Biodegradation of Reactive blue-25 by *Aspergillus ochraceus* NCIM-1146. *Bioresource Technology. 98*(18): 3638–3642. DOI: https://doi.org/10.1016/j.biortech.2006.11.017

Patel, R. J., & Bhaskaran, L. (2016). Screening of Novel Ascomycetes for the Production of Laccase Enzyme Using Different Lignin Model Compounds. *International Journal Pharmacy and Biology Science. 7*(4): 452–458. DOI: https://doi.org/10.22376/ijpbs.2016.7.4.b452-458

Ranjusha, V. P., Pundir, R., Kumar, K., & Dastidar, M. G. (2010). Biosorption of Remazol Black B dye (Azo dye) by the Growing *Aspergillus flavus*. *Journal of Environmental Science and Health. 45*(10): 1256–1263. DOI: https://doi.org/10.1080/10934529.2010.493812

Rohmawati, U., & Kasiamdari, R. S. (2022). Morphological, Molecular Characterization, and Physico-chemical Analysis of *Trichoderma yunnanense* as Indigosol Golden Yellow Dye-decolorizing Fungus. *Philippine Journal of Science. 151*(6): 2459–2470. DOI: https://doi.org/10.56899/151.6B.1

Safitri, A., Dwi Febrianti, W., & Rahmaniah, G. (2020). Effectiveness of Using *Trichoderma viride* as Biosorbent for Remazol Brilliant Purple in Batik Wastewater Treatment. *JSMARTech, 1*(2), 41–45. DOI: https://doi.org/10.21776/ub.jsmartech.2020.001.02.4

Salem, S. S., Mohamed, A. A., Gl-Gamal, M. S., Talat, M., & Fouda, A. (2019). Biological Decolorization and Degradation of Azo Dyes from Textile Wastewater Effluent by *Aspergillus niger. Egyptian Journal of Chemistry. 62*(10): 1799–1813. DOI: https://doi.org/10.21608/EJCHEM.2019.11720.1747

Senthivelan, T., Kanagaraj, J., Panda, R. C., & Narayani, T. (2019). Screening and Production of a Potential Extracellular Fungal Laccase from *Penicillium chrysogenum*: Media Optimization by Response Surface Methodology (RSM) and Central Composite Rotatable Design (CCRD). *Biotechnology Reports. 23*: 1–15. DOI: https://doi.org/10.1016/j.btre.2019.e00344

Shanmugam, S., Hari, A., Ulaganathan, P., Yang, F., Krishnaswamy, S., & Wu, Y. R. (2018). Potential of Biohydrogen Generation Using the Delignified Lignocellulosic Biomass by A Newly Identified Thermostable Laccase From *Trichoderma asperellum* Strain BPLMBT1. *International Journal of Hydrogen Energy. 43*(7): 3618–3628. DOI: https://doi.org/10.1016/j.ijhydene.2018.01.016

Singh, J., Das, A., & Yogalakshmi, K. N. (2020). Enhanced Laccase Expression and Azo Dye Decolourization During Co-interaction of *Trametes* versicolor and Phanerochaete chrysosporium. *Applied Sciences. 2*(6). DOI: https://doi.org/10.1007/s42452-020-2832-y

Singh, S., Gautam, R. L., Chaudhary, D. K., Singh, D., & Naraian, R. (2024). Biodecolorization and Biodegradation of Reactive Green 12 Textile Industry Dye and Their Post-Degradation Phytotoxicity-Genotoxicity Assessments. *Archives of Microbiology. 206*(6). DOI: https://doi.org/10.1007/s00203-024-03994-6

Sun, J., Jin, J., Beger, R. D., Cerniglia, C. E., & Chen, H. (2017). Evaluation of Metabolism of Azo Dyes and Their Effects on *Staphylococcus aureus* Metabolome. *Journal of Industrial Microbiology and Biotechnology. 44*(10): 1471–1481. DOI: https://doi.org/10.1007/s10295-017-1970-8

Zainip, V. J., Adnan, L. A., & Elshikh, M. S. (2021). Decolorization of Remazol Brilliant Violet 5R and Procion Red MX-5B by Trichoderma Species. *Tropical Aquatic and Soil Pollution. 1*(2): 108–117. DOI: https://doi.org/10.53623/tasp.v1i2.25