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DECOLORIZATION OF TEXTILE DYES WITH CRUDE LIGNINOLYTIC ENZYMES FROM *Coriolopsis caperata*

Dekolorisasi Pewarna Tekstil dengan Enzim Ligninolitik Kasar dari *Coriolopsis caperata*

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ABSTRACT
The textile industry is a major contributor to global water pollution, releasing an estimated 280,000 tons of synthetic dyes annually into aquatic ecosystems. These dyes, particularly azo compounds, are often toxic, mutagenic, and resistant to conventional wastewater treatment methods. Microbial enzymatic degradation, especially by white-rot fungi, offers a promising eco-friendly alternative. This study aimed to optimize the production of ligninolytic enzymes—Laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP)—from *Coriolopsis caperata*, and assess their efficiency in degrading two azo dyes: Reactive Red 21 (RR21) and Reactive Orange 107 (RO107). The fungus, isolated from the Peat Swamp Forest in Sebangau, Central Kalimantan, was cultured in a modified glucose-peptone medium enriched with veratryl alcohol. The optimization parameters included variations in time, dye concentration, and the addition of hydrogen peroxide (H_2O_2). Enzyme activity was quantified spectrophotometrically, and dye decolorization was assessed over time at different dye concentrations. Among the enzymes, Lac showed the highest activity (4938.05 U/L), followed by LiP (995.26 U/L) and MnP (246.47 U/L). These values notably exceed several previously reported benchmarks for fungal enzyme activity. RO107 demonstrated greater susceptibility to enzymatic degradation, with 83.71% decolorization achieved at 24 hours, while RR21 reached 65.71% at 48 hours. The addition of 1 mM H_2O_2 significantly enhanced decolorization, increasing RR21 and RO107 removal to 95.71% and 99.30%, respectively. These results underscore the oxidative synergy between H_2O_2 and ligninolytic enzymes, particularly LiP and MnP. Overall, the study demonstrates the potential scalability of *C. caperata*-based enzymatic treatment systems for textile effluent bioremediation, supporting compliance with environmental discharge regulations and contributing to sustainable wastewater management.

Keywords: *Coriolopsis caperata, decolorization, ligninolytic enzymes, textile dye*

ABSTRAK
Industri tekstil merupakan salah satu penyumbang utama pencemaran air secara global, dengan estimasi sekitar 280.000 ton zat warna sintetis dilepaskan setiap tahunnya ke dalam ekosistem perairan. Zat warna ini, terutama senyawa azo, bersifat toksik, mutagenik, dan resisten terhadap metode pengolahan limbah konvensional.

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Degradasi enzimatik oleh mikroorganisme, khususnya jamur pelapuk putih, menawarkan alternatif ramah lingkungan yang menjanjikan. Penelitian ini bertujuan untuk mengoptimalkan produksi enzim ligninolitik—lakase (Lac), mangan peroksidase (MnP), dan lignin peroksidase (LiP)—dari *Coriolopsis caperata*, serta mengevaluasi efektivitasnya dalam mendegradasi dua zat warna azo: Reactive Red 21 (RR21) dan Reactive Orange 107 (RO107). Jamur diisolasi dari Hutan Rawa Gambut di Sebangau, Kalimantan Tengah, dan dikultur dalam media glukosa-pepton termodifikasi yang diperkaya dengan veratryl alkohol. Parameter optimasi mencakup variasi waktu, konsentrasi zat warna, dan penambahan hidrogen peroksida (H_2O_2). Aktivitas enzim diukur secara spektrofotometri, dan proses dekolorisasi zat warna diamati seiring waktu pada berbagai konsentrasi. Di antara ketiga enzim, lac menunjukkan aktivitas tertinggi (4938,05 U/L), diikuti oleh LiP (995,26 U/L) dan MnP (246,47 U/L). Nilai-nilai ini melampaui beberapa data acuan sebelumnya terkait aktivitas enzim jamur. RO107 menunjukkan kerentanan lebih tinggi terhadap degradasi enzimatik dengan efisiensi dekolorisasi sebesar 83,71% dalam 24 jam, sementara RR21 mencapai 65,71% dalam 48 jam. Penambahan 1 mM H_2O_2 secara signifikan meningkatkan efisiensi dekolorisasi, masing-masing menjadi 95,71% untuk RR21 dan 99,30% untuk RO107. Hasil ini menunjukkan sinergi oksidatif antara H_2O_2 dan enzim ligninolitik, khususnya LiP dan MnP. Secara keseluruhan, penelitian ini memperlihatkan potensi skala besar dari sistem pengolahan limbah berbasis enzim *C. caperata* untuk bioremediasi limbah tekstil, mendukung kepatuhan terhadap regulasi pembuangan limbah, dan berkontribusi terhadap pengelolaan air limbah yang berkelanjutan.

Kata kunci: *Coriolopsis caperata, dekolorisasi, enzim ligninolitik, pewarna tekstil*

INTRODUCTION

The textile industry is a major contributor to environmental pollution, primarily due to the discharge of synthetic dyes, which are often non-biodegradable and toxic to aquatic ecosystems (Dutta et al. 2022; Islam et al. 2022). When released into the environment, these dyes can cause severe ecological damage, including reduced light penetration, disruption of aquatic life, and potential health hazards to both humans and animals (Tkaczyk et al. 2020; Sharma et al. 2021; Abd El-Aziz et al. 2023). Moreover, many of the breakdown products of these dyes have been linked to carcinogenic and mutagenic effects (Tkaczyk et al. 2020; Sharma et al. 2021; Abd El-Aziz et al. 2023). Despite these severe environmental and health threats, only ~20% of textile wastewater is treated, and most existing treatment methods, including coagulation, chemical oxidation, and adsorption, fail to fully degrade these toxic compounds (Abd El-Aziz et al. 2023; Khan et al. 2023). These methods often generate secondary pollutants, require high energy inputs, and incur significant operational costs, making them less sustainable for large-scale use (Abd El-Aziz et al. 2023; Khan et al. 2023). As a result, there is growing interest in environmentally friendly and sustainable alternatives for dye removal, with biological methods emerging as a promising solution (Khan et al. 2023).

Biological approaches, particularly microbial enzymatic degradation, have gained attention as an effective means for removing textile dyes (Ajaz et al. 2020; Saravanan et al. 2021; Ngo and Tischler 2022; Agnestisia et al. 2024). White-rot fungi, in particular, have been extensively studied for their ability to produce ligninolytic enzymes, which not only degrade lignin in wood cell walls but also break down complex dye molecules into less toxic or colorless byproducts (Ajaz et al. 2020; Bayineni 2021; Agnestisia et al. 2024; Pundir et al. 2024). Several white-rot fungal species, such as *Pleurotus ostreatus*, *Pleurotus eryngii*, *Phanerochaete chrysosporium*, and *Trametes versicolor*, have demonstrated effectiveness in degrading various dye classes, including azo, anthraquinone, and triphenylmethane dyes, owing to their ligninolytic enzymatic activity (Haritha and Sambasiva Rao 2009; Singh et

al. 2020; Das et al. 2023; Ge et al. 2024). These species produce enzymes such as laccase (Lac; EC 1.10.3.2), manganese peroxidase (MnP; EC 1.11.1.13), and lignin peroxidase (LiP; EC 1.11.1.14), which facilitate the degradation of a wide range of textile dyes, reducing their environmental impact (Haritha and Sambasiva Rao 2009; Kunjadia et al. 2016; Singh et al. 2020; Das et al. 2023; Aragaw et al. 2024; Ge et al. 2024; Kumar et al. 2024). Studies have shown that optimization of culture conditions can enhance the enzymatic activity and dye removal efficiency of these fungi. For instance, Ge et al. (2024) demonstrated that crude Lac from *Pleurotus eryngii* exhibited over 90% decolorization of Remazol Brilliant Blue R (RBBR) dye within 24 hours under optimized conditions (Ge et al. 2024). Similarly, Giap et al. (2022) found that LiP from *Lentinus squarrosulus* MPN12 was capable of degrading Acid Blue 281, Porocion Brilliant Blue HGR, and Acid Blue 62, achieving decolorization rates of 70.5%, 73.5%, and 92%, respectively (Giap et al. 2022). Additionally, optimization of culture media composition has been explored to enhance enzymatic activity and dye removal efficiency, as reported by (Levin et al. 2010). These findings highlight the potential of white-rot fungi and their ligninolytic enzymes as sustainable and effective decolorization agents for textile dye pollution.

Among white-rot fungi, *Coriolopsis caperata* has recently emerged as a promising candidate for bioremediation due to its notable ligninolytic activity, particularly in the production of laccases, and its ability to degrade anthraquinone and triphenylmethane dyes such as Remazol Brilliant Blue R (RBBR) and Rhodamine B (Patel et al. 2017; Mahawaththage Dona et al. 2019; Agnestisia et al. 2024). This species has shown significant potential under optimized conditions, which are influenced by factors such as pH, temperature, and enzyme concentration. Additionally, *C. caperata* has been successfully isolated from the Peat Swamp Forest at the Center for International Cooperation in Sustainable Tropical Peatland Management (CIMTROP) in Sebangau, Central Kalimantan, as reported in a previous study (Agnestisia et al. 2024), further highlighting its natural abundance and potential for dye decolorization. However, while its ability to degrade anthraquinone and triphenylmethane dyes has been well-documented, its effectiveness in degrading azo dyes—such as Reactive Red 21 (RR21) and Reactive Orange 107 (RO107)—remains unexplored. Azo dyes are a major class of synthetic dyes, with approximately 280,000 tons discharged annually, and they account for around 70% of the commercial dyes used in the textile industry (Dutta et al. 2022; Islam et al. 2022). These dyes, particularly azo compounds, are highly toxic, mutagenic, and notoriously resistant to conventional wastewater treatment methods. Their stable aromatic structure makes them difficult to degrade, posing significant environmental and health risks. The lack of studies on the degradation of azo dyes by *C. caperata* represents a critical research gap. Therefore, this study aims to fill this gap by optimizing the production of ligninolytic enzymes from *C. caperata* and evaluating its potential for degrading azo dyes, specifically RR21 and RO107. By comparing the enzyme activity and degradation efficiency of *C. caperata* with other well-studied white-rot fungi, this research will highlight the advantages of *C. caperata* as a more effective and efficient decolorization agent. The findings of this study will contribute to advancing our understanding of *C. caperata*'s enzymatic capabilities and its applicability in the decolorization of textile wastewater, offering more sustainable and environmentally friendly solutions for industrial dye removal.

MATERIALS AND METHODS

Location and time of the study

This research was conducted at the Laboratory of Biomedicine, Medical Faculty, Universitas Palangka Raya. Research activities were carried out from October 2024 to March 2025.

Materials

The *C. caperata* fungus used in this study was isolated in the previous study [8]. The ITS-rDNA sequence of this fungal isolate has been recorded in the GenBank database with the accession number PP694323. The mycelium was cultivated on

a potato dextrose agar (PDA) slant at 25 ± 2 °C and periodically sub-cultured to maintain its viability. Meanwhile, the textile dyes used in this study included Reactive Red 21 (C.I.18105) and Reactive Orange 107 (C.I.20870). All other chemicals used were of analytical grade.

Ligninolytic enzyme production

Five fungal mycelium disks (5 mm in diameter) from actively growing PDA cultures were transferred into 250 mL Erlenmeyer flasks containing 100 mL of modified glucose-peptone medium [8]. After a two-day incubation period, 1 mL of 100 mM veratryl alcohol was added to the medium to enhance ligninolytic enzyme production. The incubation duration was varied between 2 and 16 days to evaluate its effect. Following incubation, the culture media were collected and centrifuged at 5000 rpm for 20 minutes. The resulting supernatant was then used to assess ligninolytic enzyme activity and prepare a crude enzyme.

Ligninolytic enzyme activity test

The ligninolytic enzyme activities of laccase, lignin peroxidase, and manganese peroxidase were assessed using syringaldazine (Leonowicz and Gzrywnowicz 1981), veratryl alcohol (Tien and Kirk 1984), and vanillidene acetone (Paszczynski et al. 1985) as substrates, respectively. All enzymatic assays were conducted photometrically using a spectrophotometer, with three replicates for each test. The enzyme activities were expressed in nkat/mg enzyme.

The crude enzyme preparation

The collected culture medium was transferred into dialysis tubes and dehydrated with polyethylene glycol (polymerization degree: 20,000) for 6 h at 7 °C. Subsequently, the concentrated solution in the dialysis tubes was dialyzed overnight against 0.1 M phosphate buffer (pH 6.5) at 7 °C to eliminate small molecules and impurities. The resulting concentrate was then utilized as a crude enzyme preparation for subsequent analyses.

Dye decolorization at different concentrations

The decolorization assay was conducted in a 1 mL reaction system containing crude laccase (1 U/mL), dye solutions at final concentrations of 10, 20, 40, 60, 80, and 100 mg/L, and a 100 mM sodium acetate buffer (pH 5.0). The reaction mixture was thoroughly mixed, shaken at 100 rpm, and incubated in the dark at 25 ± 2 °C for 24 hours. A negative control was prepared under identical conditions using a heat-inactivated enzyme. The change in maximum absorbance before and after 24 hours of incubation was measured using a UV-visible spectrophotometer at the maximum absorption wavelength. The decolorization efficiency was calculated using the following equation:

Decolorization efficiency (%) =
$(A_0 - A_t)/A_0 \times 100\%$ …… (1)

where A_0 is the initial absorbance and A_t denotes the absorbance after decolorization process.

Dye decolorization at different time course

The decolorization assay was conducted in a 1 mL reaction system containing crude enzyme extract (1 U/mL), a dye solution at the optimum concentration for achieving the highest decolorization efficiency, 40 mM H_2O_2 and 100 mM sodium acetate buffer (pH 5.0). The reaction mixture was thoroughly homogenized, shaken at 100 rpm, and incubated at 25 ± 2 °C for 24 hours in complete darkness. Samples were collected at various time intervals (1, 3, 6, 12, 24, 36, dan 48 h) to monitor the decolorization process. The absorbance at the maximum absorption wavelength was recorded using a UV-visible spectrophotometer. The decolorization efficiency was calculated using Formula 1.

Dye decolorization by adding H_2O_2

The decolorization assay was carried out in a 1 mL reaction system comprising crude enzyme extract (1 U/mL), a dye solution at its optimal concentration, and a 100

mM sodium acetate buffer (pH 5.0). The reaction mixture was thoroughly mixed, shaken at 100 rpm, and incubated in complete darkness. Samples were taken at the optimal incubation time to assess the decolorization process. The absorbance at the dye's maximum absorption wavelength was measured using a UV-visible spectrophotometer, and decolorization efficiency was determined using Formula 1.

RESULTS AND DISCUSSION

The production of ligninolytic enzymes by *C. caperata* was successfully induced using a modified glucose-peptone medium supplemented with veratryl alcohol. The addition of veratryl alcohol, a well-known inducer of ligninolytic enzyme synthesis, has been reported to enhance the activity of ligninolytic enzymes, particularly in white-rot fungi (Thakkar and Bhatt 2020; Mahdy and Suttinun 2023). In this study, the enzyme production was assessed over an incubation period ranging from 2 to 16 days, allowing for the determination of optimal conditions for enzyme synthesis.

Figure 1 illustrates that incubation time plays a crucial role in enzyme yield, aligning with previous research demonstrating that prolonged cultivation enhances enzyme secretion until peak production is achieved (Suboh et al. 2022; García-Martín et al. 2024). After incubation, the fungal culture was centrifuged to separate the mycelium from the extracellular enzyme-rich supernatant, a widely used method for obtaining crude enzyme extracts, as ligninolytic enzymes are predominantly secreted into the surrounding medium (Kumari et al. 2019). The resulting crude extract was subsequently analyzed for enzymatic activity to quantify the production of Lac, LiP, and MnP. The enzyme activity assay results revealed distinct yet interrelated activity patterns throughout the incubation period. Lac demonstrated the highest enzymatic activity, peaking at 516.77 U/L on day 12, followed by a decline, likely due to nutrient depletion or the accumulation of inhibitory metabolites. This finding aligns with previous research indicating that Lac is a predominant enzyme in many white-rot fungi, facilitating the early-stage oxidation of organic compounds (Eichlerová and Baldrian 2020; Hadibarata and Yuniarto 2020; Suryadi et al. 2022). The early peak in Lac activity suggests its primary role in initiating lignin degradation and other oxidative reactions in the fungal metabolism. In contrast, LiP exhibited a more prolonged activity profile, with peak activity (206.73 U/L) recorded on day 16. This aligns with studies on *Phanerochaete chrysosporium* and *Trametes versicolor*, where LiP showed extended enzymatic activity, contributing to the breakdown of more recalcitrant lignin structures over time (Dashtban et al. 2010; Cui et al. 2021; Alzabaidi et al. 2025). Similarly, MnP activity peaked at 52.77 U/L on day 16, further supporting the notion that MnP and LiP contribute to sustained oxidative transformations, particularly under conditions where laccase activity declines. The variations in peak activity and production timelines observed in *C. caperata* suggest a sequential and coordinated enzymatic mechanism, where Lac plays a dominant role in early-stage oxidation, while LiP and MnP facilitate subsequent oxidative transformations. This pattern is consistent with findings from other white-rot fungi, emphasizing that different enzymes are regulated based on substrate availability, metabolic conditions, and environmental factors (Kumar and Chandra 2020). Moreover, the prolonged activity of LiP and MnP suggests their importance in maintaining oxidative potential in later stages of organic compound degradation, which may be critical for dye decolorization.

On the molecular level, the differences in activity between these ligninolytic enzymes—Lac, LiP, and MnP—can be attributed to their distinct catalytic structures, substrate specificities, and regulatory patterns during enzyme production. Although lignin degradation did not occur in this study since the fungus was cultured in a glucose-peptone medium supplemented with veratryl alcohol, the enzymes were still differentially expressed in a time-dependent manner. Lac, a multicopper oxidase, has three types of copper centers (Type 1, 2, and 3) that facilitate efficient electron transfer from phenolic substrates to molecular oxygen, generating water and reactive oxygen species (ROS) (Janusz et al. 2020; Gałązka et al. 2023). This structure enables Lac to rapidly

oxidize a wide range of simple aromatic compounds, which accounts for its early activity peak at day 12. In contrast, LiP contains a heme group and depends on H_2O_2 to form a high-valent iron-oxo intermediate (compound I) capable of oxidizing complex aromatic molecules like veratryl alcohol, even in the absence of lignin (Hiner et al. 2002). MnP, which oxidizes Mn^{2+} to Mn^{3+} in the presence of H_2O_2, requires manganese ions for optimal functioning (Hofrichter 2002; Manavalan et al. 2015), though its activity was detected due to trace elements in the medium. Both LiP and MnP peaked later at day 16, indicating continued oxidative enzyme production under prolonged incubation. These findings suggest that even without lignin as a substrate, the induction and expression of ligninolytic enzymes in *C. caperata* can be effectively stimulated by veratryl alcohol and regulated by metabolic timing, allowing the fungus to prepare its enzymatic system for potential oxidative challenges.

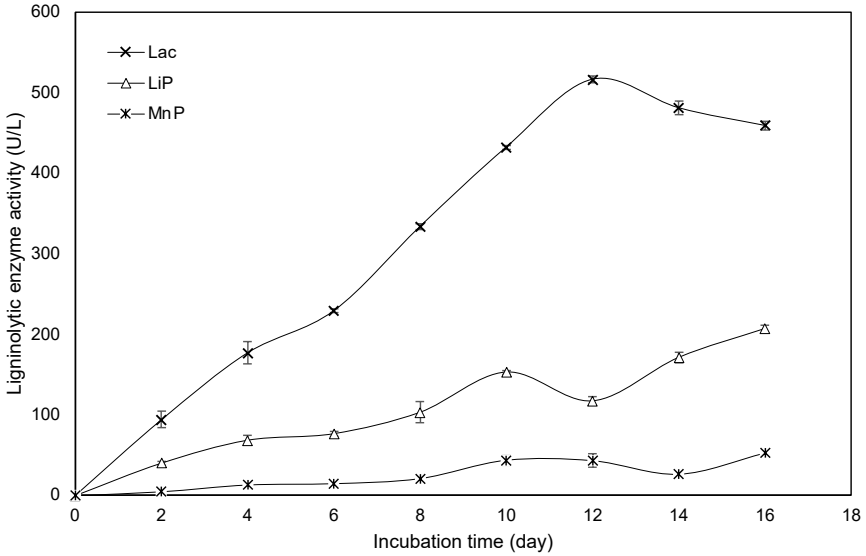

Figure 1. Ligninolytic enzyme activities at different incubation times

Following enzyme production, the preparation of crude enzyme extracts is crucial for dye decolorization assays, as it ensures enzyme stability and activity. The efficiency of enzyme purification directly influences the reliability of subsequent enzymatic assays In this study, the culture medium containing extracellular enzymes was transferred into dialysis tubes and dehydrated using polyethylene glycol (PEG) with a polymerization degree of 20,000. PEG dehydration has been widely recognized as an efficient method for increasing enzyme concentration without exposing the sample to high temperatures that could lead to protein denaturation (Ingham 1984). The controlled dehydration at 7°C for 6 hours effectively removed excess water while maintaining enzyme stability, a key factor in ensuring enzymatic functionality. To further purify the enzyme solution, the concentrated extract underwent dialysis overnight against 0.1 M phosphate buffer (pH 6.5) at 7°C. This process was essential for removing small molecules and impurities that could interfere with enzyme assays (Dako et al. 2012). Dialysis has been extensively used in enzyme purification studies, with previous research demonstrating its effectiveness in removing salts, metabolites, and other low-molecular-weight contaminants (Dako et al. 2012; Kavya et al. 2019). Maintaining a low temperature throughout the purification steps was crucial for preserving enzymatic integrity, preventing denaturation, and enhancing the reliability of subsequent analyses. Ultimately, the purified enzyme concentrate was utilized for dye decolorization assays. Previous research has shown that enzyme purity and concentration directly impact the efficiency of dye degradation.

The application of dye decolorization serves as an effective method to evaluate the oxidative potential of ligninolytic

enzymes, providing valuable insights into their catalytic efficiency and potential applications in wastewater treatment. The impact of this preparation process on enzyme activity was evident in the observed significant increases in enzyme activity levels. Laccase activity exhibited the most substantial increase, rising from 516.77 ± 4.39 U/L before preparation to 4938.05 ± 14.82 U/L after preparation. Similarly, lignin peroxidase activity increased from 206.73 ± 4.82 U/L to 995.26 ± 18.68 U/L, while manganese peroxidase activity rose from 52.77 ± 1.97 U/L to 246.47 ± 10.64 U/L (Table 1).

Table 1. Ligninolytic enzyme activity before and after crude enzyme preparation

Enzyme	Ligninolytic enzyme activity (U/L)	
	Before preparation	After preparation
Laccase	516.77 ± 4.39	4938.05 ± 14.82
Lignin Peroxidase	206.73 ± 4.82	995.26 ± 18.68
Manganese Peroxidase	52.77 ± 1.97	246.47 ± 10.64

The significant enhancement in enzyme activity after preparation suggests that the applied concentration and purification methods effectively enriched the enzyme content and removed potential inhibitors. Statistical analysis using one-way ANOVA revealed a highly significant difference in enzyme activity levels among the three enzymes after preparation ($F = 14{,}179.43$, $p < 0.001$). This finding underscores the distinct enzymatic efficiencies of laccase, LiP, and MnP, highlighting their different catalytic roles in oxidative processes. The remarkable increase in laccase activity suggests its predominant role in degradation, consistent with previous studies that emphasize its high oxidative potential. Meanwhile, the moderate increases in LiP and MnP activity confirm their complementary functions in ligninolytic pathways, contributing to the breakdown of complex organic compounds. The prepared enzyme concentrates were subsequently used in dye decolorization assays to evaluate their oxidative potential. By optimizing the enzyme preparation process, the efficiency of ligninolytic enzymes can be maximized. Overall, the findings demonstrate that the combined use of PEG dehydration and dialysis is an effective strategy for obtaining highly active crude enzyme extracts.

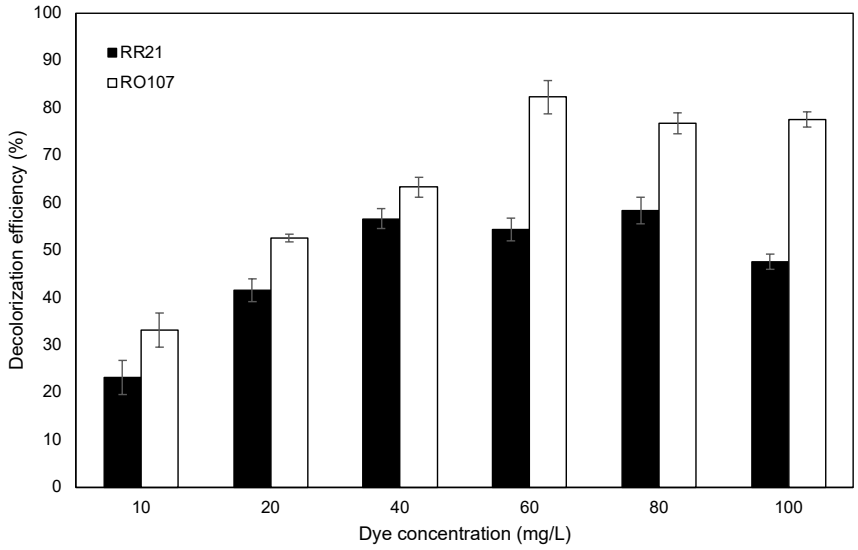

Figure 2. Decolorization efficiency of RR21 and RO107 at different dye concentrations using ligninolytic enzymes

The effectiveness of enzymatic dye decolorization is influenced by several factors, including enzyme and dye concentrations, incubation time, and reaction condition (Singh et al. 2015). In this study, the decolorization efficiency of ligninolytic enzymes was assessed using two distinct dyes, Reactive Red 21 (RR21) and Reactive Orange 107 (RO107), at varying concentrations (10–100 mg/L) in a sodium acetate buffer (pH 5.0) at 25 ± 2 °C over a 48-hour period. The results indicated significant differences in decolorization efficiency between the two dyes, suggesting variability in their susceptibility to enzymatic degradation (Figure 2). For RR21, decolorization efficiency increased with concentration up to 80 mg/L, reaching a peak of 58.39% before slightly decreasing to 47.62% at 100 mg/L. This optimal decolorization at 80 mg/L likely reflects the ideal enzyme-substrate interaction. The observed decrease in decolorization efficiency at higher dye concentrations, particularly at 100 mg/L, can be attributed to enzyme saturation, accumulation of toxic by-products, and decreased substrate accessibility, highlighting the need to optimize enzyme and dye concentrations for maximum efficiency (Agnestisia et al. 2024). In contrast, RO107 exhibited higher overall decolorization efficiency than RR21 at all concentrations. The efficiency increased steadily with dye concentration, peaking at 82.38% at 60 mg/L, and unlike RR21, RO107 maintained a relatively high decolorization rate (77.62%) even at 100 mg/L, suggesting a greater enzymatic susceptibility. When comparing both dyes, ligninolytic enzymes showed superior decolorization efficiency for RO107 at all concentrations. This indicates that RO107 has a more accessible molecular structure for enzymatic degradation compared to RR21, with differences in dye composition, such as the presence of specific functional groups, potentially facilitating more effective enzymatic breakdown (Kumar et al. 2024).

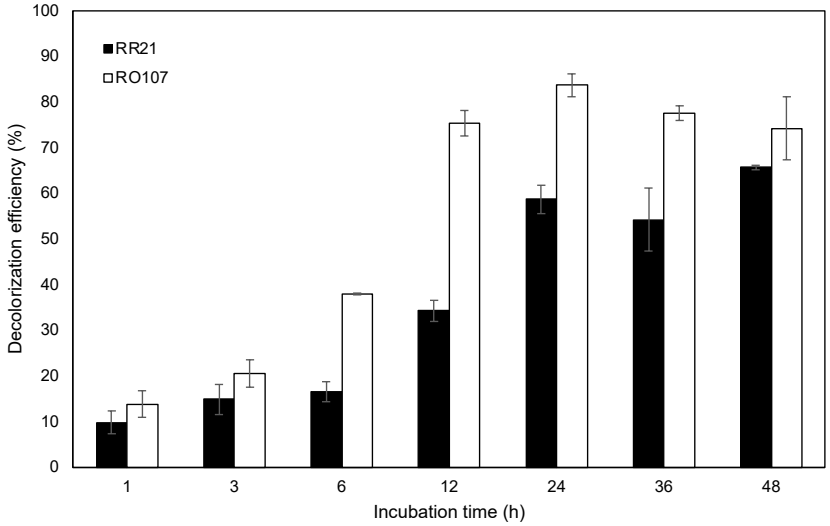

Figure 3. Decolorization efficiency of RR21 and RO107 at different time incubation using ligninolytic enzymes

The incubation time analysis of dye decolorization also used in this study for providing valuable insights into the enzymatic degradation kinetics and efficiency of the crude enzyme extract. In this study, decolorization efficiency was monitored at different time intervals (1, 3, 6, 12, 24, 36, dan 48 hours) to determine the optimal incubation period for maximum dye removal. Both dyes showed an increasing trend in decolorization efficiency over time, but the rate and extent of decolorization varied significantly between them (Figure 3). According to previous studies, the enzymatic degradation process is often characterized by a slow initial phase due to enzyme activation and the establishment of substrate-enzyme interactions (Noor et al. 2013). For RR21, the de-

colorization process exhibited a gradual increase, reaching 34.38% at 12 hours and peaking at 65.71% at 48 hours. The initial phase (1–6 hours) showed relatively low decolorization efficiency (9.90–16.71%), likely due to enzyme activation and substrate-enzyme interactions. A significant increase occurred between 12 and 24 hours, suggesting optimal enzymatic conditions and substrate availability. However, a slight decline at 36 hours (54.29%) before reaching a maximum at 48 hours may indicate potential enzyme inhibition or substrate depletion. In contrast, RO107 exhibited a much higher and more rapid decolorization rate, reaching 75.38% within just 12 hours and peaking at 83.71% at 24 hours. This result suggests that RO107 is more susceptible to enzymatic degradation than RR21, possibly due to differences in molecular structure. Unlike RR21, which required 48 hours to reach peak decolorization, RO107 achieved near-complete degradation within 24 hours. However, after reaching its peak, decolorization efficiency slightly declined at 36 and 48 hours (77.62% and 74.29%, respectively), which could be attributed to enzyme instability, by-product accumulation, or substrate exhaustion. Comparing both dyes, RO107 consistently showed higher decolorization efficiency than RR21 across all time points, suggesting that its molecular structure is more accessible to enzymatic attack. This difference may be due to variations in dye composition, such as the presence of functional groups that facilitate enzymatic breakdown. The slower degradation of Remazol Red indicates a more complex structure that requires prolonged incubation for effective degradation.

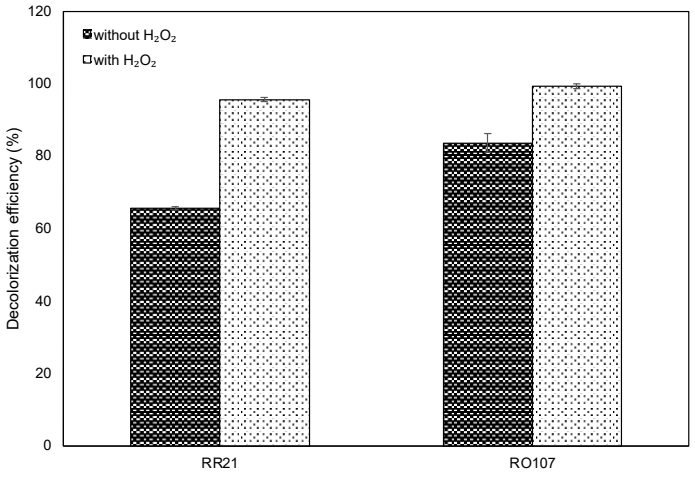

Figure 4 Decolorization efficiency of RR21 and RO107 in the presence and absence of H_2O_2

The addition of hydrogen peroxide (H_2O_2) was evaluated for its effect on dye decolorization using ligninolytic enzymes under optimized reaction conditions. The results revealed that H_2O_2 significantly enhanced the decolorization efficiency of both RR21 and RO107 dyes (Figure 4). H_2O_2 plays a crucial role in dye degradation by serving as a co-substrate for peroxidase enzymes, such as LiP and MnP, leading to the generation of reactive oxygen species (ROS) (Ferreira-Leitão et al. 2007; Kumar and Chandra 2020). Upon activation by these enzymes, H_2O_2 undergoes heterolytic cleavage, forming high-valent iron-oxo intermediates (e.g., Compound I in LiP), which initiate oxidative reactions (Hiner et al. 2002). In MnP-mediated reactions, H_2O_2 drives the oxidation of Mn^{2+} to Mn^{3+}, forming diffusible oxidants (Hofrichter 2002; Manavalan et al. 2015). These ROS—hydroxyl radicals (•OH), superoxide anions ($O_2^{\bullet -}$), and high-valent metal-oxo species—attack the chromophoric structures of synthetic dyes. The interaction between ROS and dyes primarily targets conjugated π-electron systems and auxochromes such as azo, anthraquinone, and triphenylmethane groups, which are responsible for the dyes' color (Mielgo et al. 2003). Oxidative cleavage of double bonds or aromatic rings by ROS results in decolorization. In this study,

decolorization efficiency increased for both RR21 and RO107 in the presence of H_2O_2, with RR21 reaching 95.71% and RO107 nearly achieving complete decolorization at 99.30%. The higher decolorization efficiency observed for RO107 suggests its molecular structure is more accessible to enzymatic degradation. In the absence of H_2O_2, RR21 decolorization reached 75.71%, while RO107 showed a higher efficiency of 83.71%, further supporting the hypothesis that RO107 is more amenable to enzymatic degradation. The presence of H_2O_2 significantly boosted decolorization efficiency, demonstrating its crucial role in enhancing enzymatic dye breakdown. However, it is important to consider the environmental impact of hydrogen peroxide. At high concentrations, H_2O_2 can be toxic and may deplete dissolved oxygen levels in aquatic environments, potentially harming aquatic life. The increased oxidative stress from excessive H_2O_2 can disrupt the chemical balance of water bodies, affecting microbial communities and overall ecosystem health (Bilbao-garcía and Duoandicoechea 2025). Therefore, it is essential to carefully optimize H_2O_2 concentrations to balance its benefits in enhancing enzymatic activity with potential environmental risks. Proper monitoring and regulation of H_2O_2 levels during decolorization processes will be necessary to minimize adverse effects while maximizing dye degradation efficiency.

The effectiveness of ligninolytic enzymes in dye degradation depends heavily on their catalytic structures, which determine substrate specificity, redox potential, and degradation efficiency. Lac, LiP, and MnP each possess unique active sites that give them distinct biochemical roles. Lac, a multicopper oxidase, features three copper centers (Types 1, 2, and 3) that enable it to catalyze the oxidation of phenolic substrates, reducing molecular oxygen to water (Janusz et al. 2020; Aza and Camarero 2023). Its moderate redox potential (0.5–0.8 V) allows it to degrade simpler phenolic dyes effectively but limits its action on more complex structures unless redox mediators are present (Frasconi et al. 2010). LiP, containing a heme group, forms highly reactive intermediates upon activation by hydrogen peroxide, enabling it to oxidize non-phenolic aromatic compounds with a higher redox potential (~1.2 V) (Martínez 2002). MnP, also using a heme cofactor, oxidizes Mn^{2+} to Mn^{3+}, which forms complexes that can indirectly oxidize phenolic and non-phenolic substrates (Martínez 2002; Martinez et al. 2013). These enzymes work together in a sequential and complementary manner during dye degradation, with Lac initiating the process and LiP and MnP sustaining activity on more resistant compounds. Compared to other white-rot fungi, *C. caperata* exhibits superior enzymatic performance, with Lac activity reaching 4938.05 U/L, surpassing that of *Trametes versicolor* (Jing et al. 2007) and *Pleurotus ostreatus* (Baldrian and Gabriel 2002). Its LiP and MnP activities (995.26 U/L and 246.47 U/L, respectively) also outperformed those of *Coriolopsis gallica* (Elisashvili et al. 2017) dan *Phanerochaete chrysosporium* (Singh and Pakshirajan 2010), translating into high dye decolorization efficiencies, with RO107 reaching 99.30% and RR21 95.71% when supplemented with H_2O_2. The combination of advanced catalytic properties and high enzyme activity highlights the potential of *C. caperata* enzymes for environmentally friendly and efficient bioremediation of dye-contaminated effluents.

Furthermore, the use of crude enzyme extracts, which exhibit higher enzyme activities, resulted in greater substrate binding and catalytic efficiency, leading to more effective oxidative degradation of dye molecules. This was particularly important for complex dyes such as RR21 and RO107, which require sustained enzymatic activity for complete degradation. The decolorization data clearly demonstrated that the use of purified enzymes resulted in significantly higher removal rates—up to 99.30% for RO107 and 95.71% for RR21 in optimized conditions with H_2O_2. The correlation between crude enzyme preparation and dye degradation underscores the importance of enzyme quality in decolorization applications, as purified enzymes not only reduce the time required for decolorization but also allow for more complete breakdown of dye structures, minimizing the accumulation of toxic intermediates. Thus, crude enzyme preparation plays a critical role in enhancing the environmental effectiveness and

operational feasibility of enzymatic dye treatment systems.

CONCLUSIONS

This study demonstrated the potential of *C. caperata* as an effective bioremediation agent for textile dye pollution through its ligninolytic enzyme activity. Lac exhibited the highest enzymatic activity, followed by LiP and MnP, indicating its dominant role in dye degradation. The enzymatic decolorization assays revealed that RO107 was more susceptible to enzymatic breakdown than RR21, with RO107 achieving an 83.71% decolorization rate within 24 hours, compared to 75.71% for RR21 at 48 hours. Furthermore, the addition of hydrogen peroxide (H_2O_2) significantly enhanced the decolorization process, increasing the efficiency of RR21 to 95.71% and RO107 to 99.30%. This improvement is attributed to H_2O_2's role in enhancing ligninolytic enzyme activity, particularly LiP and MnP. These findings highlight the effectiveness of *C. caperata* in degrading azo dyes and underscore its potential for sustainable wastewater management in the textile industry. Future research should focus on optimizing environmental conditions for enzyme production and purification, as well as enhancing color removal efficiency to improve practical applicability.

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