JURNAL KIMIA VALENSI
p-ISSN: 2460-6065; e-ISSN: 2548-3013
Journal homepage: https://journal.uinjkt.ac.id/index.php/valensi
Research Article

Decolorization of Reactive Black 5 by *Myceliophthora thermophila* KLUM$_1$

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Article Info
Received: Marc 25, 2025
Revised: May 16, 2025
Accepted: May 26, 2025
Online: May 31, 2025

Citation:
Susanti, E., Jati, R. F., Apriani, D., Suharti., & Alvionita, M. (2025). Decolorization of Reactive Black 5 by *Myceliophthora thermophila* KLUM$_1$. *Jurnal Kimia Valensi*, 11(1), 81-91.

Doi: 10.15408/jkv.v11i1.45573

Abstract
This research aimed to ascertain the impact of alkaline lignin of teak sawdust on the synthesis of ligninase and decolorization of Reactive Black 5 (RB5) by *M. thermophilia* KLUM$_1$, also to elucidate the mechanism of mycoremediation. This research was conducted within the cultivation of the spore suspension in a co-culture setting. The RB5 dye percentage decolorization and ligninase activity were produced by varying the addition of alkaline lignin. The results show that the addition of 0.5% alkaline lignin accelerates the production of ligninase in the Kirk medium. However, adding more than 0.5% alkaline lignin does not affect the highest amount of ligninase produced. The increase in RB5 dye decolorization by *M. thermophila* KLUM$_1$ is proportional to the increase in alkaline lignin in the medium. The percentage of wood-rot fungi decolorization in Kirk medium with and without lignin with the addition of 0.5%, 1%, and 2% lignin on the 10th day was 26.29%, 29.51%, 34.74%, and 40.43%, respectively. The decolorization process of RB5 by *M. thermophilia* KLUM$_1$ in Kirk medium with alkaline lignin occurs through several mechanisms, i.e., enzymatic degradation, adsorption by the growing mycelia, and alkaline lignin present in the medium.

Keywords: Alkaline lignin, decolorization, ligninase, *Myceliophthora thermophilia* KLUM$_1$, reactive black 5

1. INTRODUCTION

Wood-rot Fungi (WRF) constitute microorganisms that demonstrate superior degradation capabilities of synthetic dyes compared to bacteria. The degradation of dyes by WRF is dependent on the ligninase system under aerobic conditions, whereas bacteria utilize the enzyme azoreductase under anaerobic conditions [1]. WRF is capable of producing extracellular enzymes in the form of an oxidative ligninase system comprising lignin peroxidase (LiP), manganese peroxidase (MnP), and phenol oxidase (laccase). These oxidative systems are known to degrade dyes into CO_2 and H_2O, whereas bacteria reduce azo dyes into aromatic amine compounds that are toxic [2]. Consequently, the application of bacteria for dye decolorization is typically conducted in an anaerobic environment, followed by an enzymatic or aerobic stage to facilitate the degradation of aromatic amine compounds into harmless substances [3]. Additionally, WRF possesses the advantage of rapid growth and resilience to extreme conditions [4].

The white-rot fungus *Myceliophthora thermophilia* KLUM$_1$, isolated from weathered cocoa shell waste [5], belongs to the phylum Ascomycetes. It has leafy hyphae, short conidiophores resembling branching stalks, and conidia. The conidia are round or elliptical in shape, yellow in color, with thick walls, and easily detachable. They are produced by a mycelium that is flush with the media and has a top surface that is brownish-white. The colonies are rough to the touch and have a texture similar to cotton. The Ascomycetes phylum is known to possess the capacity to produce ligninase enzymes and decolorize synthetic dyes [6]. *M. thermophilia* KLUM$_1$ has been identified as a promising source of ligninase enzymes based on capacity to degrade methylene blue dye [5], the information of distinct zones in selective media containing methylene blue serves as clear evidence of

this process. Additionally, it has been observed to decolorize a range of synthetic dyes, including rhodamine B, methylene blue, Reactive Black 5 (RB5), reactive blue HEGN, reactive red HE7B, and reactive yellow HE4R [7]. The production of the ligninase enzyme is known to be proportional to its decolourization ability towards synthetic dyes [8].

Several growth conditions, including temperature, pH, time, nitrogen source, carbon source, humidity, and oxygen levels influence the production of ligninase enzymes [9]. The most common temperature range for fungi growth is between 30 and 37 degrees Celsius. The pH of the medium exerts a dual influence on the growth conditions of the fungi and the production and activity of metabolites. This is due to the effect of pH on cell permeability [9]. The sources of carbon and nitrogen exert a considerable effect upon the growth of fungi and the production of ligninase enzymes. The utilization of limited conditions has been observed to increase the production of ligninase enzymes. Such enhancement can be attributed to the utilization of carbon sources derived from organic materials containing lignin, such as sawdust, straw, and corn stover [10]. Optimal humidity can facilitate the physiological conditions necessary for fungi growth. In the process of lignin degradation, an adequate oxygen level is also required for oxidative reactions to occur. Therefore, it is essential to consider these factors in order to increase the production of ligninase enzymes.

The production of ligninase with a carbon source analogous to its natural substrate, alkaline lignin derived from teak sawdust, yielded a higher specific activity than that observed with the carbon source of glucose. The specific activity values of enzymes produced from teak sawdust as a carbon source (LiP 55.65 U/mg, MnP 52.48 U/mg, and laccase 57.64 U/mg) and glucose (LiP 60.67 U/mg, MnP 46.56 U/mg, and laccase 23.28 U/mg) were obtained [9]. The subsequent study demonstrated that alkaline lignin derived from diverse types of sawdust, including teak, jackfruit, and acacia wood, influences the production of laccase enzyme activity and the rate of decolorization of Reactive Black 5 by *Thermothelomyces guttulata* $KLUM_2$. The highest specific activity of the laccase enzyme, cultivated on Kirk's medium with teak wood alkaline lignin as the carbon source, was observed to be 93.39 ± 25.09 U/mg. Additionally, the greatest rate of RB5 decolorization was also achieved on Kirk's medium with teak wood alkaline lignin, reaching 21.13% with a dye concentration of 15 ppm and 0.2 grams of alkaline lignin [11]. These findings indicate that alkaline lignin derived from teak sawdust is an effective carbon source for the production of ligninase enzymes. Nevertheless, the study has not examined the impact of varying lignin concentrations on the observed outcomes; therefore, this study aims to explain this and the mechanism that occurs. The objective of this study was to determine the impact of varying quantities of alkaline lignin derived from teak sawdust on the production of ligninase enzyme and the mechanism of decolorization of Reactive Black 5 synthetic dye by *M. thermophilia* $KLUM_1$ in an alkaline teak wood lignin medium.

2. RESEARCH METHOD

Suspension Preparation of *Myceliophthora thermophilia* $KLUM_1$

Cultures of *M. thermophilia* $KLUM_1$ were grown on potato dextrose agar (PDA) slant agar at 37° C for 14 days. Following the incubation period, the spores were resuspended using a 0.02% Tween-80 solution. The suspension was then subjected to a 10-minute vortexing process, after which it was left to stand for an additional 30 minutes. Subsequently, the suspension was filtered using sterile glass wool or cotton in a sintering apparatus, resulting in the production of a sterile spore suspension. The filtrate is a suspension of *M. thermophilia* $KLUM_1$, with the cell density determined through the measurement of optical density at a wavelength of 660 nm. Cell density ($\sum_{cell}/mL_{spore\ suspension}$) is determined using Equation 1.

The standard OD660 nm for *M. thermophilia* $KLUM_1$ was 0.281 with a cell density in the spore suspension based on hemocytometer of 24,4 x 10^6 cell/mL[12].

Effect of Lignin Alkaline from Teak Wood Sawdust on Ligninase Production and Decolorization of Synthetic Dye Reactive Black 5 by *Myceliophthora thermophilia* $KLUM_1$

A total of 2.1×10^7 spore cells of *M. thermophilia* $KLUM_1$ were inoculated in 20 mL of Kirk's medium with an alkaline lignin carbon source derived from teak sawdust. The samples were prepared with concentrations of alkaline lignin in 0.5%, 1.0%, and 2.0%, and the addition of the synthetic dye Reactive Black 5 at 35 mg/L (ppm). They were then incubated for 10 days at 37°C. Control samples were prepared using Kirk's medium without the addition of dye or alkaline lignin. Kirk's production medium lacking alkaline lignin was designated 'RB5', the Kirk's production medium lacking dye was designated 'alkaline lignin', and Kirk's production medium containing dye and alkaline lignin was designated 'RB5 + alkaline lignin'. Each treatment was subjected to centrifugation at 3000 rpm daily. The resulting supernatant was evaluated for its decolorization potential, pH, and enzyme activity. The pH value was determined using a pH meter. The extent of dye decolourization was quantified using a

visible light spectrophotometer at a wavelength of 600 nm. Percentage decolourization represents the reduction in colour intensity resulting from decolourization by wood-rot fungi, calculated using the Equation (2 and 3).

$$\sum_{cell}/mL_{spore\ suspension} = \frac{OD_{660\ measurement}}{OD_{660\ standart}} \times standard\ cell\ density\ \left(\frac{cell}{mL}\right) \quad \text{.....(1)}$$

$$\%\ \text{decolourization "RB5"} = \frac{(A_{RB5}-A_{Blank})_0 - (A_{RB5}-A_{Blank})_n}{(A_{RB5}-A_{Blank})_0}\ x\ 100\% \quad \text{.....(2)}$$

$$\%\ \text{decolourization "RB5 + Lignin"} = \frac{(A_{RB5+lignin}-A_{lignin})_0 - (A_{RB5+lignin}-A_{lignin})_n}{(A_{RB5+lignin}-A_{lignin})_0} x100\% \quad \text{.....(3)}$$

Where:

A_{RB5} = absorbance of supernatant variation without lignin alkaline
A_{Blanko} = absorbance of supernatant variation without lignin alkaline and dye
$A_{RB5 + Lignin}$ = absorbance of supernatant variation with lignin alkaline and dye
A_{Lignin} = absorbance of supernatant variation with lignin alkaline and without dye

Determination of Adsorption of Reactive Black 5 Dye of *Myceliophthora thermophilia* KLUM$_1$ Fungal Mycelia in the RB5 Decolorization Process in 10 Days

The adsorption of the RB5 dye by fungi mycelia was evaluated through the extraction of the dye adhered to the mycelia using methanol in accordance with the established principles. The residue obtained from the production of 'RB5 + 1% Alkaline Lignin' and 'RB5' was extracted with 10 mL of 70% methanol using fungi mycelia, incubated at 37 °C for 24 hours, and then centrifuged at 3000 rpm for 10 minutes. The supernatant was then measured for absorbance using a UV-Vis spectrophotometer at a wavelength of 600 nm. The quantity of dye absorbed by the fungal mycelium was calculated using the following equation (4).

$$\%\ \text{Adsorption by the fungal mycelium} = \frac{\left(A_n\ x\ \frac{1}{2}\right)}{A_0}\ x\ 100\% \quad \text{.....(4)}$$

Where:

A_n = absorbance at n-days
A_0 = absorbance at 0-days
1/2 = dilution factor (10 mL ethanol/20mL Kirk medium)

Determination of Adsorption of Reactive Black 5 Dye by Alkaline Lignin in RB5 Decolorization Process in 10 days

The adsorption of the Reactive Black 5 (RB5) dye by alkaline lignin was determined by adding the dye at a concentration of 35 mg/L (ppm) and varying the concentration of alkaline lignin. The variations were as follows: the samples were prepared by adding 0.5%, 1.0%, and 2.0% of the dye into Kirk's medium and incubating them at 37 °C. Subsequently, the mixtures were centrifuged at 3000 rpm for 10 minutes. The supernatant was then measured for absorbance using a visible light spectrophotometer at the maximum absorption wavelength of RB5, which is 600 nm. The quantity of dye absorbed by alkaline lignin was calculated using the following equation (5).

$$\%\ \text{Lignin Alkaline Adsorption} = \frac{A_0 - A_n}{A_0}\ x\ 100\% \quad \text{...(5)}$$

Where:

A_n = absorbance at n-days
A_0 = absorbance at 0-days

Lignin Peroxidase (LiP) Assay

LiP enzyme activity was determined by the addition of 0.8 mL of a 10 mM veratryl alcohol solution to a test tube. Subsequently, 1mL of 0.2 M tartaric acid and 1.5 mL of distilled water were added. Additionally, 0.2 mL of the enzyme crude extract and 0.5 mL of 50 mM H_2O_2 were introduced. Subsequently, the absorbance was detected at a wavelength of 310 nm at the initial time point (minute 0) and one minute after the addition of H_2O_2 to the solution. The LiP enzyme activity can be determined from the resulting absorbance value using equation (6), with a molar excitation coefficient value of εmax = 9300 $M^{-1}cm^{-1}$[113].

$$Enzyme\ Activity\ \left(\frac{U}{ml}\right) = \frac{(At-A0)\times V_{tot}\times 10^6}{\varepsilon_{max}\times d\times V_{\ enzyme}\times t} \text{.....(6)}$$

Where:

A_0 = absorbance at minute 0
A_t = absorbance at minute 1
V_{tot} = total volume of solution (ml)
ε_{maks} = molar excitation coefficient value
d =cuvette thickness (cm)
V_{enzim} = volume of enzyme crude extract (ml)
t = time (menit)

Manganese Peroxide (MnP) Assay

MnP enzyme activity was determined by preparing a solution of 0.5 mL of pH 5.5 0.2 M acetate buffer, 0.8 mL of 1 mM guaiacol, 1 mL of 20 mM citric acid, 0.5 mL of 50 mM H_2O_2, and 1.5 mL of 0.1 mM $MnSO_4$. Additionally, 0.2 mL of the enzyme crude extract was incorporated into the mixture. The mixture was then permitted to stand for a period of 15

minutes. The activity of MnP was determined based on the amount of guaiacol oxidized, and the resulting absorbance was measured at a maximum wavelength of 465 nm at both the 0^{th} and 1^{st} minute. The activity of the MnP enzyme can then be determined using equation (6) with the value of the molar excitation coefficient $\varepsilon max = 12100\ M^{-1}cm^{-1}$.

Laccase Assay

Laccase enzyme activity was determined based on its ability to oxidize guaiacol, as previously described. The oxidation of guaiacol was quantified spectrophotometrically at a wavelength of 470 nm. A total of 0.8 mL of a 10 mM guaiacol solution was combined with 0.8 mL of a 0.2 M acetate buffer at pH 5. Additionally, 0.2 mL of the enzyme crude extract was introduced. Subsequently, the absorbance was measured at a wavelength of 470 nm at the 0^{th} and 1^{st} minutes. The laccase enzyme activity can be determined from the resulting absorbance value by means of equation (6), taking the molar excitation coefficient value of $\varepsilon max = 6740\ M^{-1}cm^{-1}$.

3. RESULT AND DISCUSSION

Effect of Lignin Alkaline Addition on Ligninase Enzyme Activity during RB5 Dye Decolourization Process by *Myceliophtora thermophilia* $KLUM_1$

Lignin peroxidase (LiP) is the most readily expressed ligninase enzyme by *M. thermophilia* $KLUM_1$ in comparison to other ligninase enzymes, namely manganese peroxidase (MnP) and laccase. The activity of the LiP enzyme was observed in the medium containing 0.5 and 1% alkaline lignin from the second day after inoculation. In contrast, the medium lacking alkaline lignin demonstrated the onset of this activity on day 6. This result is in accordance with the findings of previous research[14], which indicated that ligninase activity by *M. thermophilia* $KLUM_1$ in Kirk's medium commenced an increase on day 6. The highest activity of all ligninase enzymes (LiP, MnP, and laccase) was observed in all variations between days 6 and 7, after which a decrease was observed on days 8 to 10. This is likely due to the fact that the addition of alkaline lignin as a carbon source precipitates the formation of limited nutrient conditions. Ligninase is an inducible enzyme, and therefore only produced in limited medium conditions[15]. The utilisation of lignin as the sole carbon source in this study facilitated the formation of limited nutrient conditions and expedited the generation of the ligninase enzyme in a manner that was more rapid than that observed in the absence of lignin. Moreover, the decline in ligninase activity may be attributed to the reduction in nutrient levels, which may precipitate the onset of the death phase in the cells. The result of this study is also analogous to the profile of ligninase production by *P. chrysosporium*, which began measurement on day 4 and exhibited the highest ligninase activity on day 6, followed by a decline on days 7 to 10[16].

The addition of lignin resulted in an elevated level of MnP production in comparison to the control medium devoid of lignin supplementation (**Figure 1A**). However, the addition of lignin did not lead to an increase in the amount of LiP and laccase (**Figure 1**). In light of the findings of this study, it can be concluded that the incorporation of 0.5% teak wood lignin alkaline into Kirk's medium has the effect of accelerating the production of LiP and increasing the production of MnP by *M. thermophilia* $KLUM_1$ (**Figure 1B**). However, the addition of lignin alkaline over 0.5% does not appear to influence the maximum amount of ligninase produced (**Figure 1C** and **Figure 1D**).

Effect of Lignin Alkaline on the Decolorization of RB 5 Dye by *Myceliophthora thermophilia* $KLUM_1$

The percentage of RB5 decolorization by *M. thermophilia* $KLUM_1$ demonstrated an increase from day 2 to day 10 in all variations, both in the medium with the addition of alkaline lignin (0.5%, 1%, and 2%) and in the absence of alkaline lignin. The decolorization percentage of RB5 exhibited an increase in proportion to the quantity of alkaline lignin introduced to the medium. There was a notable surge in the percentage of decolorization on day 5, which was followed by a gradual increase on subsequent days. This outcome was observed to be consistent across all instances of alkaline lignin addition. The addition of 0.5% alkaline lignin on day 5 resulted in a decolorization percentage of 12.43%, which increased to 24.86% by day 6. The addition of 1% alkaline lignin resulted in a percentage of decolourization of 19.86% on day 5, which increased to 30.66% on day 6. Upon the addition of 2% alkaline lignin, the percentage of decolorization on day 5 was 23.88%, and this increased by 34.82% on day 6 (**Figure 2**).

Figure 1 illustrates a correlation between ligninase enzyme production and the percentage of RB5 dye decolorization. The increase in ligninase enzyme production and the increase in RB5 decolorization occurred concurrently, specifically during the growth period from day 5 to day 6. However, this was not the case in the data from days 7 to 10. During this period, the production of the ligninase enzyme decreased, while the percentage of decolorization remained constant and even exhibited a slight increase. It is hypothesized that the mechanism of Reactive Black 5 decolorization by *Myceliophthora thermophilia* $KLUM_1$ is not solely reliant on ligninase enzyme activity. Additionally, the adsorption of RB 5 dye by $KLUM_1$ fungal mycelia and the adsorption of RB 5 by alkaline lignin may also play a role[17,18,19].

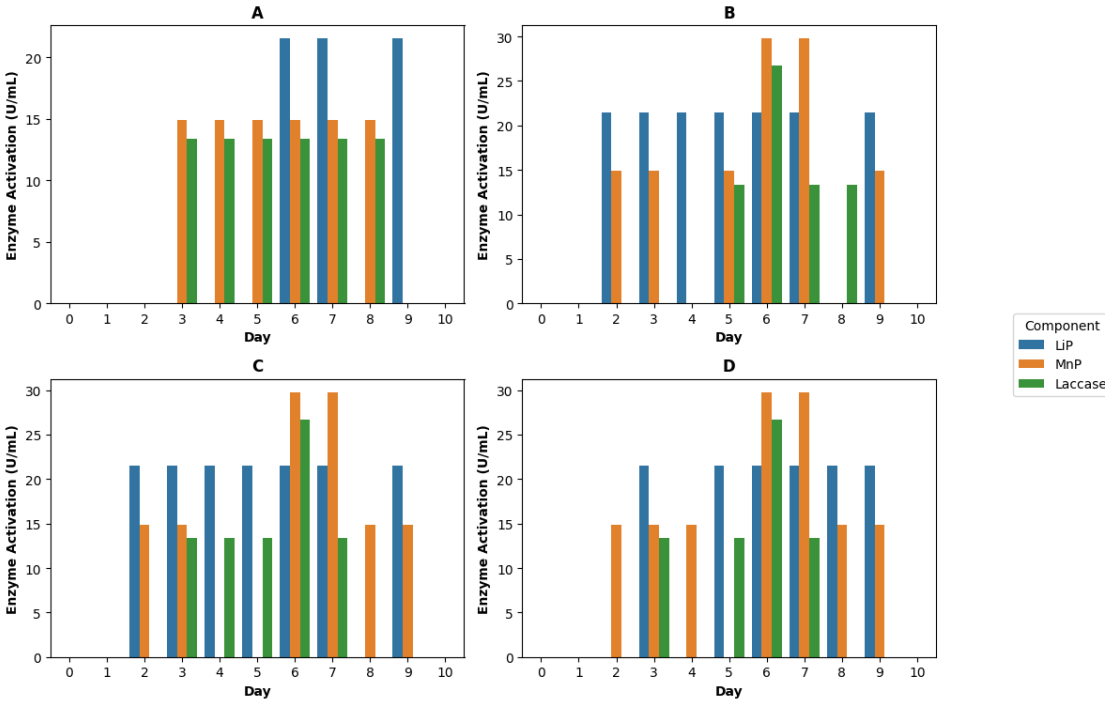

Figure 1. Enzyme activity graph (A) variation of 'no alkaline lignin + RB5' (B) variation of '0.5% alkaline lignin + RB5' (C) variation of '1% alkaline lignin + RB5' (D) variation of '2% alkaline lignin + RB%'

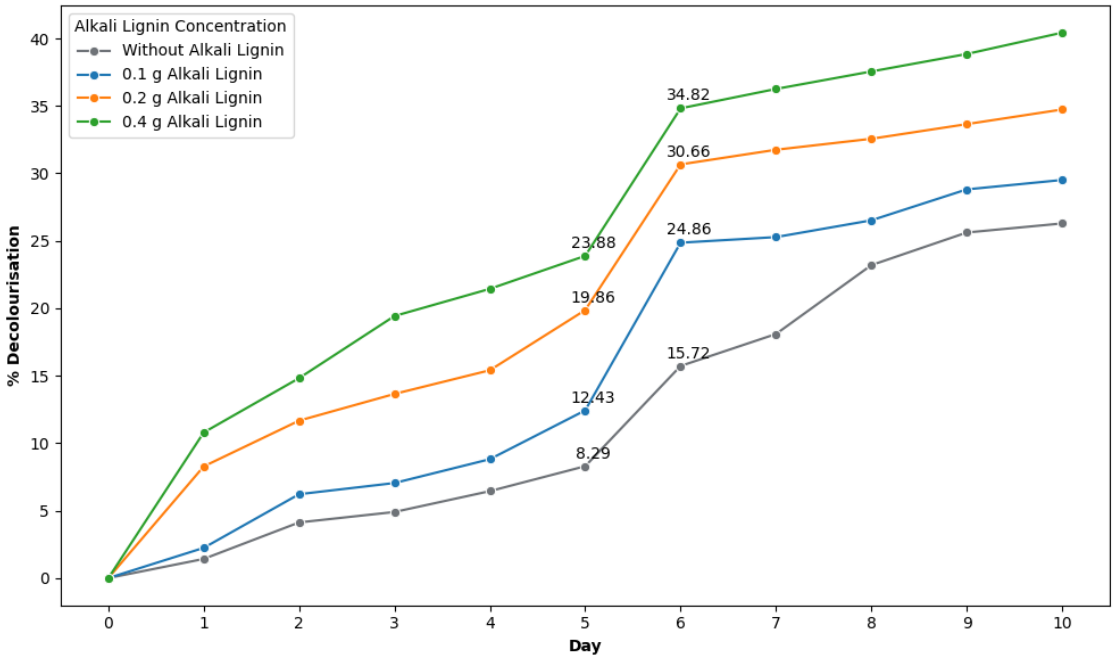

Figure 2. Decolourization of RB5 by *Myceliophthora thermophilia* KLUM$_1$ in medium with the addition of different amounts of lignin alkaline (0.5%, 1%, and 2%)

The increase in % decolorization from alkaline lignin variations (0.5%; 1%; and 2%) at the longest incubation time of 10 days was observed to increase to 29.51%; 34.74%; and 40.34%, respectively (**Figure 2**). The control media, lacking alkaline lignin, exhibited a decolorization percentage of only 26.29%, a value consistent with that observed in previous research. This earlier study [7] demonstrated that the decolorization process of RB5 by *M. thermophilia* KLUM$_1$ in Kirk's medium utilizing a glucose carbon source yielded a percentage of 24.99%. These findings suggest that the incorporation of alkaline teak wood sawdust lignin may enhance the decolorization of RB5 dye by *M. thermophilia* KLUM$_1$. These findings are consistent with those of research [20], which demonstrated that the addition of lignin to *Trametes hirsuta* isolates could enhance laccase activity and the decolorization capacity of Remazol Brilliant Blue R

(RBBR) by 82.66% to 99.29%. These findings are supported by other studies, including research[21] on the decolorization process of RB 5 dye with a concentration of 100 mg/L by the three fungal isolates (BRB 81, BRB 11, BRB 73) on alkaline lignin media, with rates of 77.8%, 21.5%, and 51.3%, respectively. As evidenced by research [17], the decolorization rate of DRB5 dye by the *Ganoderma lucidum* EN2 isolate can be enhanced by up to 95.16% with the addition of 0.60 g/L alkaline lignin within 48 hours. A study[22] demonstrated that *Bjerkandera sp.* isolate BOL 13, when cultured with biomass waste (lignin) as a carbon source, exhibited enhanced efficiency in decolorizing Remazol Red dye, with a 65-90% increase in efficacy when cultured for 12 days. The research[18] indicated that the bio decolorization of azo dyes by *Echinodontium taxodii* was enhanced when the fungus was cultured with lignin, presumably it because phenylpropane units as building blocks of lignin through free radical reactions produce low molecular weight products that can act as mediators for peroxidase or laccase enzymes [22].

Adsoprtion of Reactive Black 5 Dye by *Myceliophthora thermophilia* KLUM$_1$ Fungal Mycelia and Alkaline Lignin

In order to substantiate the hypothesis that the decolorization of Reactive Black 5 by *M. thermophilia* KLUM$_1$ occurs via the process of dye adsorption by fungal mycelia and alkaline lignin, a comparison was conducted between the adsorption capacity of KLUM$_1$ isolates that had been inoculated in a medium with the addition of 1% alkaline lignin and those that had not been inoculated in such a medium. The results demonstrated that the adsorption process of RB5 by *M. thermophilia* KLUM$_1$ in the medium containing alkaline was more pronounced than in the medium lacking lignin (**Figure 3**).

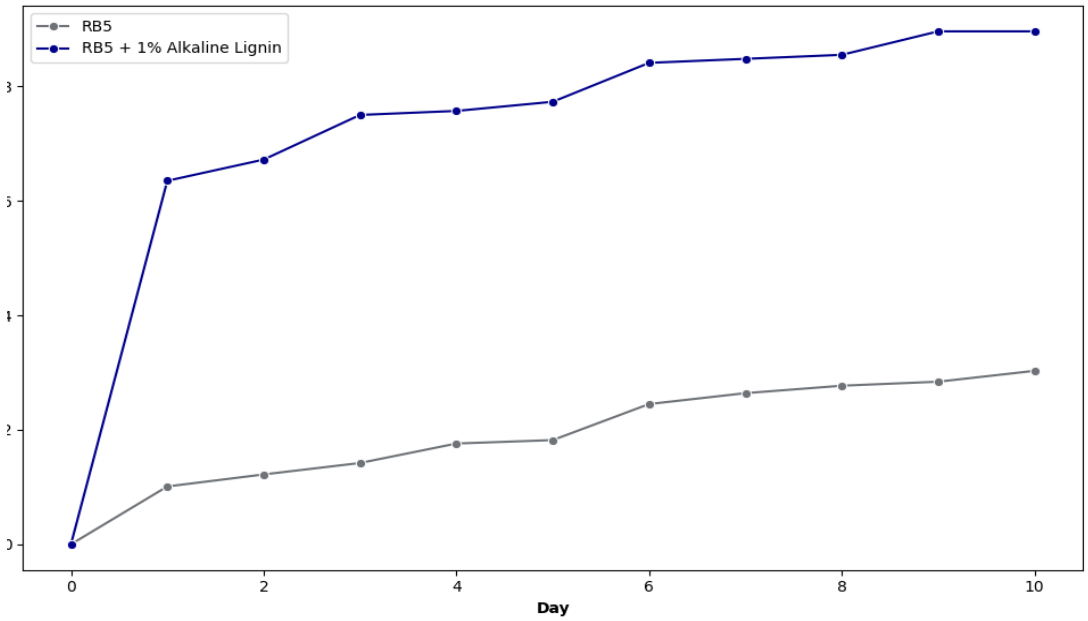

Figure 3. Adsorption rate of RB5 dye by *Myceliophthora thermophilia* KLUM$_1$ mycelium grown in medium without alkaline lignin (squares) and addition of 1% alkaline lignin (triangles)

The wood rot fungi wall is composed of polysaccharide acids, amino acids, and lipids, which contain a variety of functional groups, including amino acids, carboxyls, thiols, and phosphates [19]. The cell wall component of the wood rot fungi mycelium has positively charged groups (amino groups), while the RB5 has negatively charged functional groups (sulfonate groups). The opposite polarity of these groups results in an electrostatic attraction force between the positively charged groups on the cell wall and the negatively charged groups on the RB5, causing the latter to be adsorbed to the former [23]. Furthermore, the physical structure of wood-rot fungi mycelia is characterized by a porous texture and a rough surface, which facilitates the adsorption and absorption of the dye. The results of this experiment support the hypothesis that the decolorization of RB5 by wood rot fungi is not solely due to the degradation of the dye structure by the peroxidase enzyme produced but also due to the adsorption of RB5 on the cell wall of the wood rot fungi mycelium.

3.1. Adsorption of Reactive Black 5 by Alkaline Lignin

The findings indicated that an enhancement in the quantity of alkaline lignin resulted in a corresponding increase in the percentage of adsorption of RB5, as shown in **Figure 4**. The porous and fibrous

structure of alkaline lignin teak sawdust enables it to absorb synthetic dyes, including RB5, effectively. Furthermore, the interaction between RB5 dye and alkaline lignin can occur from the formation of hydrogen bonding between the two substances. Hydrogen atoms form bonds with oxygen or nitrogen atoms of molecules that possess higher electronegativity. The difference in electronegativity between hydrogen atoms (H) and oxygen (O), nitrogen (N), or fluorine (F) atoms in different molecules gives rise to the phenomenon of hydrogen bonding. The hydrogen atoms that bond with oxygen, nitrogen, or fluorine atoms exhibit a partial positive charge (δ-) due to enhanced electron attraction. Research indicates that teak sawdust lignin is a reactive compound, possessing functional groups including hydroxyl, methoxyl, carbonyl, and phenyl propane groups [24]. Alkaline lignin teak sawdust contains hydroxyl (-OH) groups (di-aryl propane unit), which allow hydrogen (H) atoms to participate in hydrogen bonding with oxygen (O) atoms on sulfonate (SO_3H) present in RB5 dye (**Figure 5**). The formation of these hydrogen bonds results in a robust interaction between alkaline lignin and RB5 dye, which in turn influences the efficiency and capacity of alkaline lignin in adsorbing RB5 dye.

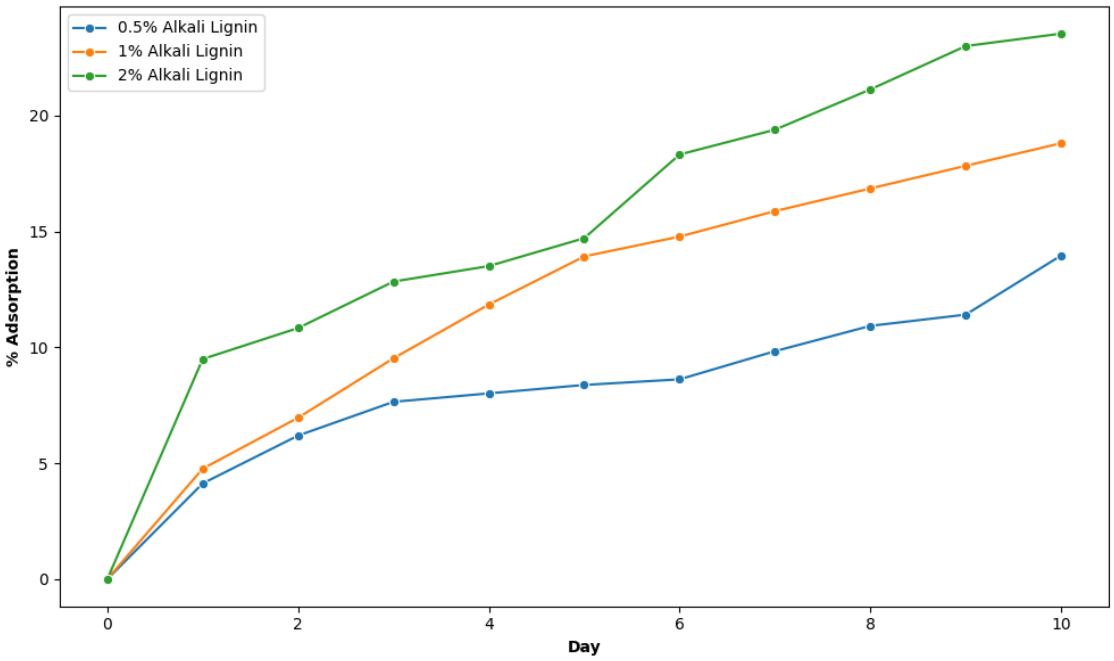

Figure 4. Adsoprtion rate of RB5 Dye with Alkaline Lignin Addition of 0.5%; 1% and 2% Without Spore Suspension

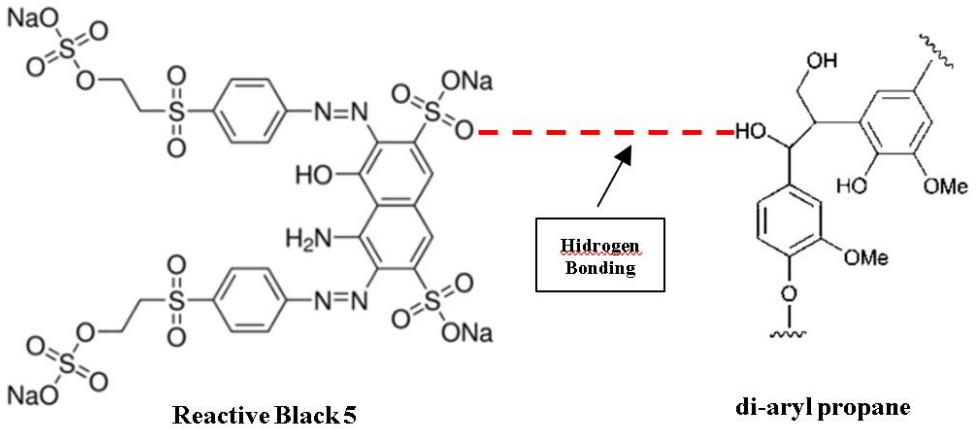

Figure 5. One of hydrogen bonding between Reactive Black 5 and unit of lignin (di-aryl propane)

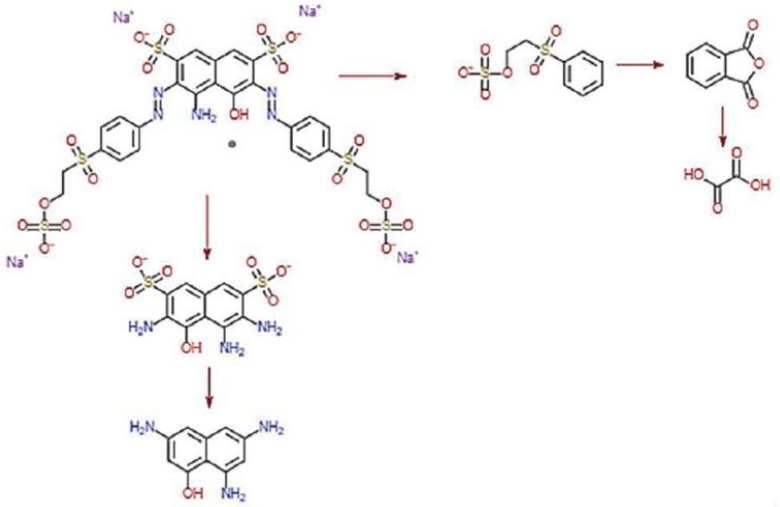

Figure 6. Catalytic mechanism of reactive black 5 by peroxidase enzyme[26]

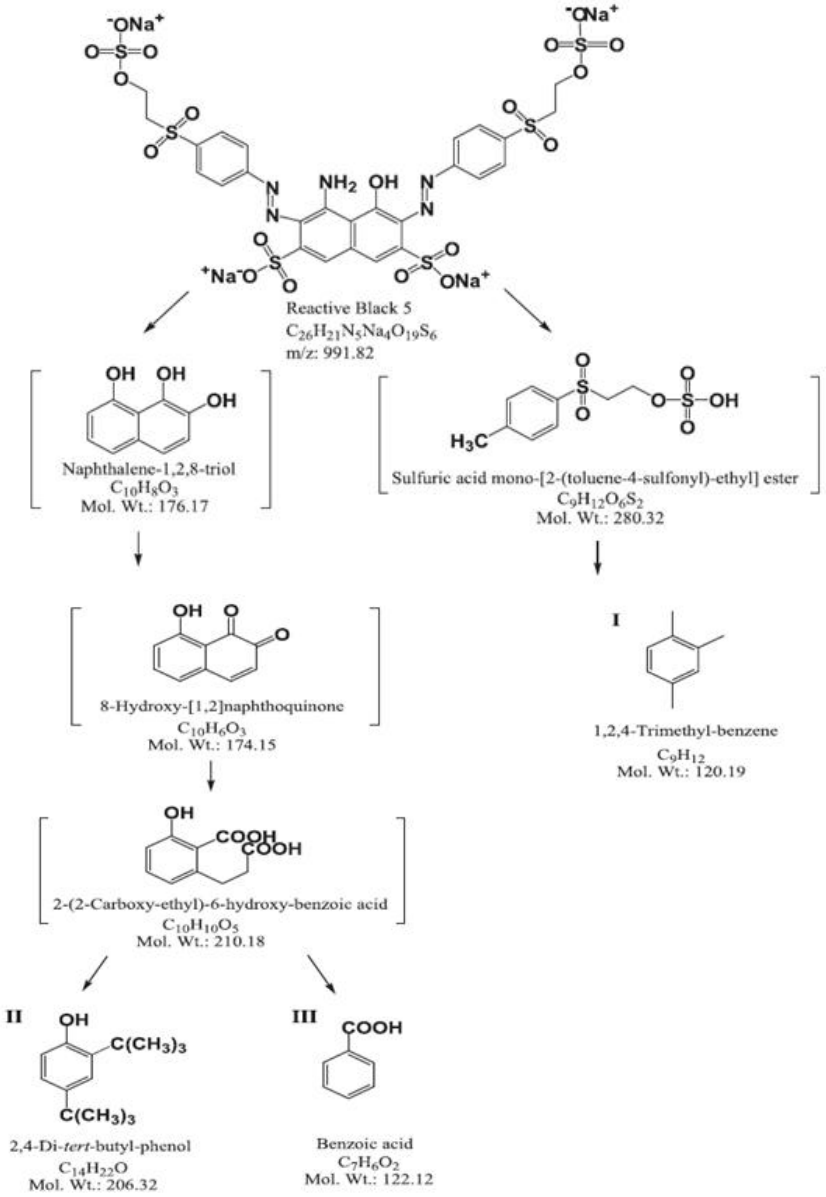

Figure 7. Biodegradation of RB5 by laccase of *Trichoderma atroviride* F03[27]

This result is similar to the findings of previous research [25]. The adsorption capacity of beech wood sawdust to methylene blue dye at a concentration of 1.4±14 mg/L is 3.05%. Research [18] indicates that the decolorization of azo dyes by *Echinodontium taxodii* is due to the adsorption of dyes by alkaline lignin, with an adsorption amount of less than 10%. Research [19] indicates that the adsorption of alkaline lignin in the process of decolorization of Alizarin Blue Black B dye by *H. heamatococca* K37 fungi can enhance the decolorization of dye wastewater, with the adsorption by alkaline lignin estimated to be between 20 and 25%. These findings suggest that alkaline lignin may also contribute to the decolorization of RB5 in a medium containing lignin, as explained in **Figure 5**. Practically, this research can be applied to textile industry waste treatment technology to reduce environmental contamination and support ecosystem sustainability.

Based on the experiment shows that the decolorization process of RB5 by *M. thermophilia* $KLUM_1$ in Kirk's medium with an alkaline carbon source of teak sawdust lignin occurred through several mechanisms, namely enzymatic degradation by ligninase (lignin peroxidase, mangan peroxidase, and lakase), adsorption RB5 by growing mycelia and adsorption RB5 by alkaline lignin. Peroxidase enzymes can degrade synthetic dyes by involving hydrogen peroxidase as an oxidizer. When H_2O_2 interacts with the active side of the peroxidase enzyme, it results in the formation of hydroxyl radicals ($OH\cdot$). The hydroxyl radicals will react with RB5 and form free radicals in RB5 compounds that have one or more unbonded electrons that make them reactive. The peroxidase enzyme oxidizes the azo bond and sulfonate group, resulting in the breaking of the bis azo N=N bond to 3,4,6-triamino-5 hydroxynaphthalene-2,7-disulfonate. Then, desulfonation of the sulfonate group to isobenzofuran-1,3-dione and HOOC-COOH (oxalic acid) occurs (**Figure 6**) [26]. According to research [27], the degradation mechanism of RB 5 by Ascomycete as wood-rot fungi (WRF) *Trichoderma atroviride F03* with laccase enzyme starts with bond breaking, followed by deamination and hydroxylation using laccase mediators. Biodegradation starts with bis-azo bond cleavage, followed by deamination and hydroxylation using laccase mediators, which results in naphthalene-1,2,8-triol and sulfuric acid mono-[2-(toluene-4-sulfonyl)-ethyl] ester. The loss of amino groups ($-NH_2$) probably occurs through deamination and hydroxylation processes, which are catalyzed by the enzyme laccase as the main mechanism in degrading the substrate. Furthermore, desulfonation of sulfuric acid mono-[2-(toluene-4-sulfonyl)-ethyl] ester occurs, resulting in the formation of 1,2,4-trimethyl-benzene. The study proved that the biodegradation mechanism of RB5 proceeds with the fission of the aromatic ring of naphthalene-1,2,8-triol, where the oxidized ring at positions C1 and C2 is broken down into 2-(2-carboxy-ethyl)-6-hydroxy-benzoic acid through the formation of 8-hydroxy-[1,2]-naphthoquinone. RB5 is further degraded through two possible pathways: (i) through decarboxylation and methylation to 2,4-di tert-butyl-phenol, and (ii) converted to benzoic acid through a decarboxylation mechanism (**Figure 7**).

4. CONCLUSION

The findings of the research indicated that the incorporation of 0.5% teak wood lignin alkaline into Kirk's medium facilitated the production of LiP and enhanced the production of MnP by *M. thermophilia* $KLUM_1$. However, the introduction of lignin alkaline over 0.5% did not influence the maximum amount of ligninase produced. The increase in RB5 dye decolorization by *M. thermophilia* $KLUM_1$ was found to be proportion to the increase in alkaline lignin amount in the medium. The percentage of decolorization of wood-rot fungi in Kirk's medium without lignin, and Kirk's medium with 0.5%, 1%, and 2% lignin addition on day 10 were 26%, 29.01%, 29.51%, 34.74%, and 40.43% respectively. The decolorization process of RB5 by *M. thermophilia* $KLUM_1$ in Kirk's medium with an alkaline carbon source of teak sawdust lignin occurred through several mechanisms, namely enzymatic degradation by the enzyme ligninase produced, adsorption by growing mycelia and adsorption by alkaline lignin.

ACKNOWLEDGMENTS

Thanks to the Institute for Research and Community Service (LPPM), State University of Malang, for supporting this research through the Grant for Publication of Thesis Results of UM Non-APBN Internal Funds in 2024 with contract number 4.4.152 / UN32.14.1 / LT / 2024.

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