https://doi.org/10.48048/tis.2024.7610

Potential of White Rot Fungi from Berbak-Sembilang National Park, Indonesia for Decolorization and Detoxification Commercial Direct Dyes

Ike Apriani[1,2], Dede Heri Yuli Yanto[3], Poedji Loekitowati Hariani[4,*], Hary Widjajanti[5] and Oktan Dwi Nurhayat[3]

[1]*Doctoral Program, Faculty of Mathematics and Natural Science, Universitas Sriwijaya, Sumatera Selatan 30139, Indonesia*
[2]*Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Raden Fatah Palembang, Sumatera Selatan 30452, Indonesia*
[3]*Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Cibinong 16911, Indonesia*
[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Sumatera Selatan 30139, Indonesia*
[5]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Sumatera Selatan 30139, Indonesia*

([*]Corresponding author's e-mail: puji_lukitowati@mipa.unsri.ac.id)

Received: 15 October 2023, Revised: 25 November 2023, Accepted: 2 December 2023, Published: 10 April 2024

Abstract

Commercial direct dyes are frequently employed in the dyeing of Jumputan, a traditional craft originating from South Sumatra, Indonesia. The introduction of synthetic dyes into the environment can have a detrimental impact on ecosystem stability, necessitating the implementation of remedial measures. There has been a growing interest in the utilization of White Rot Fungi (WRF) as a viable biological agent for the purpose of decolorizing and detoxifying synthetic colors. However, it is imperative to investigate the isolated WRF from Berbak-Sembilang National Park (TNBS) due to the varying capacities of decolorization and detoxification exhibited by each WRF. The study involved 5 WRF from TNBS screened in solid media, and grown in a liquid medium containing commercial direct dyes (direct turquoise, direct orange and direct yellow). WRF grown on liquid medium contains a single dye, mixture dyes and temperature characterization. It measured decolorization, biomass and enzyme activities. Toxicity is measured by the BSLT method. The findings indicated that 3 specific fungi, namely *Phellinus noxius* (BRB 11), *Lentinus sajor-caju* (BRB 12) and *Leotrametes menziesii* (BRB 73), exhibited a significant capacity to remove the color from both individual and mixture of direct dyes through the production of laccase and MnP enzymes. The optimal temperature for decolorization of the mixture of direct dyes was 35 °C for BRB 11 (61.4 %) and BRB 73 (60.7 %), whereas BRB 12 (47.4 %) exhibited optimal temperature at 30 °C. The toxicity assay conducted using *A. salina* showed a progressive rise in the LC_{50} value, from 15.37 mgL^{-1} in the control group to a range of 21.63 - 35.84 mgL^{-1} in the treated group, indicating a detoxification process. However, the most toxic isolate was BRB 12. This study proposes the potential of 2 isolates, *Phellinus noxius* (BRB 11) and *Leotrametes menziesii* BRB 73, from TNBS for degradation of single and mixture dye wastewater in the environment.

Keywords: Decolorization, Berbak-Sembilang National Park, White rot fungi, Detoxification, Laccase, MnP

Introduction

Jumputan, a traditional craft originating from South Sumatra, Indonesia, often incorporates commercial direct colors in its manufacturing process. The dyes in question are categorized as azo dyes, characterized by the presence of azo groups (-N=N-) [1]. This particular category has a propensity for fast dermal absorption, hence increasing the potential for allergic responses, ocular irritation and carcinogenic effects [2]. Dyes are categorized as micro pollutants and have the potential to be identified in aquatic ecosystems at concentrations as little as 1 mgL^{-1}. There exists a potential for it to be included in the food chain [3]. The introduction of dyes into the environment has a significant impact on the overall stability of the ecosystem, necessitating the use of specialized treatment methods prior to their entry into the ecosystem [4].

In recent times, there has been an increasing inclination towards the use of white rot fungus (WRF) for the purpose of decolorizing colors [5]. The potential use of this fungus in the remediation of dyes has considerable potential owing to its ecologically friendly characteristics and cost-efficiency [4,6]. Additionally, a number of groups have the capacity to effectively eradicate contaminants without negatively impacting the environment. The user has provided a numerical reference [7].

Berbak-Sembilang National park in Indonesia has a diversity of WRF, which has been reported to have the ability to decolorize synthetic dye (RBBR, AB129, AO7 and RB5), hence demonstrating potential for application in wastewater treatment processes [8]. The decolorization capacity of the WRF strain exhibits variability. The genus *Trametes* has been documented to possess a broad range of capabilities for dye biodegradation [5]. *Trametes hirsuta* D7 isolated from peat areas in Riau was reported to degrade Acid blue 129, RBBR, Reactive Black 5, acid orange 7 and methyl violet [9]. *Trametes* sp. EDN084 isolated from North Sumatra can degrade RBBR, AB 25, Reactive Blue 4, RB 5 and Reactive Red 120, Acid Blue 113 and Direct Blue 71 [10]. *Trametes* sp. AS03 isolated from mangrove forest in Riau can degrade, Levafix E3GA (Or64), Sumifix S., Scarlet 2GF (R222), Levafix B. Red E-6BA (R159) and Remazol B. Violet [11].

WRF is reported to carry out decolorization by biosorption, biodegradation or a combination of both [12]. The degradation mechanism of dye involves enzymes, both intracellular and extracellular. Enzymes produced by WRF are ligninolytic enzymes, such as manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Lac) enzymes [13] that have been related to their ability to degrade natural polymers and different synthetic chemicals, usually recalcitrant to biodegradation [14].

Enzymes can degrade the molecular structure of dye, resulting in the formation of less complex molecules. They can attach to the cell wall or be secreted outside to form a synergistic degradation system. [15]. In some cases, when azo dyes are degraded, the metabolite products formed are more toxic than the parent dye molecules. The toxicity of the end product of degradation depends on the enzyme complexes produced by microbes, the microbial environment conditions and the dyes' chemical structure [16]. Toxicity assessment for aquatic toxicity of textile dyes and dye-containing effluents can use a bioassay approach using *Artemia salina* as an animal model [17].

There is a dearth of literature pertaining to the decolorization and detoxification of commercially available direct dyes, such as direct yellow (DY), direct turquoise (DT) and direct orange (DO), using WRF strains that are isolated from TNBS. This gap in research encompasses both the treatment of individual dyes as well as the simultaneous treatment of dyes mixture. The present study included a total of 5 potential isolates of WRF, specifically identified as BRB 11, BRB 12, BRB 73, BRB 74 and BRB 81. Thus, this study aims to investigate WRF isolated from Berbak-Sembilang National Park (TNBS) which has potential in decolorization and detoxification of direct commercial dyes

Materials and methods

Materials

The materials used in this study encompass malt extract broth, glucose (himedia), $CuSO_4$ (Wako), a mixture of commercial direct dyes (direct turquoise, direct orange and direct yellow), NaOH, HCl, peptone (Himedia), MEA (Merck), acetate buffer, ABTS (Sigma Aldrich), malonic buffer, DMP (Merck), $MnSO_4$ (Merck) and H_2O_2 (Merck). Fungal isolates, namely *Phellinus noxius* (BRB 11), *Lentinus sajor-caju* (BRB 12), *Leotrametes menziesii* (BRB 73), *Flavodon flavus* (BRB 74) and *Ceriporia lacerate* (BRB 81) were obtained from the culture collection of Research Center for Applied Microbiology at BRIN.

Methods

Screening isolates on solid medium

The fungi isolates were inoculated on Malt extract agar (MEA) and grown at 25 ± 3 °C for 7 days. Mycelia plug (diameter: 5 mm) from each isolate was then sterilely moved onto a double-layer agar medium. This medium contained a mixture of commercial direct dyes at a concentration of 500 mgL^{-1} and was incubated for 7 days at temperatures between 25 and 30 °C. The formulation for the double-layer medium adhered to the method outlined by Anita *et al.* [9]. Throughout these 7 days, measurements were taken daily to record the decolorization levels and growth diameter of the fungi (in cm/day).

Decolorization mixture commercial direct dyes by WRF isolates

Fungal isolate cultures were grown on MEA at 25 ± 3 °C temperature for 7 days. Three plugs from the fungal isolates (diameter: 5 mm) were then cultured in 20 mL of Malt Glucose Peptone (MGP) broth (containing malt at 20 gL^{-1}, peptone at 1 gL^{-1} and glucose at 20 gL^{-1}) for 7 days at temperatures ranging from 25 to 30 °C [18]. The commercial direct dye mixture was introduced into the fungal culture until it reached a final concentration of 100 mgL^{-1}. A control was set up without any fungus present in the culture. The supernatant from the fungal culture was centrifuged at 10.000 rpm, at room temperature for 10 min for decolorization assay [19]. However, supernatant directly used for laccase activity, and manganese activity. Observations were made after 24, 48, 72 and 96 h incubation periods. Each experiment was performed 3 times.

Decolorization single commercial direct dye by selected isolates

The procedure for this step is the same as the previous decolorization procedure. However, the dye used is a single commercial direct dye (DY, DT and DO) up to a concentration of 100 mgL^{-1}.

Characterization of selected isolates in liquid medium containing a mixture of commercial direct dyes at various temperature

Fungal the procedure for this step is the same as the previous decolorization procedure. However, incubated at varying temperatures (30, 35, 40 and 45 °C).

Toxicity assay

Toxicity was determined using the Brine Shrimp Lethality Test (BSLT) [17]. *Artemia salina* cysts were suspended in natural seawater and incubated under 60 Watts lamp at an ambient temperature of 24 - 26 °C for 48 h. Ten larvae were put in a container containing a mixture of direct dye with varying concentrations (5, 10, 15, 20 and 25 %) and negative control without dyes. It was carried out in triplicates. Mortality (%) was calculated after 24 h of incubation using the following equation. LC_{50} value was calculated based on the probit intercept log of treatment concentration and mortality (%). Mortality was calculated according to Eq. (1).

$$\text{Mortality (\%)} = \frac{\text{Mortality before treatment} - \text{Mortality after treatment}}{\text{Mortality before treatment}} \times 100\ \% \quad (1)$$

Y = ax + b
Y = constant probit 50, a = variable x, b = intercept

Decolorization assay

Decolorization assay using a TECAN infinite 200 pro microplate reader (Switzerland) in wavelength value interval of 400 - 700 nm. Percentage of decolorization was calculated according to Eq.(2) [19].

$$\text{Decolorization (\%)} = \frac{(\text{A0} - \text{A1})}{\text{A0}} \times 100 \quad (2)$$

A0 = the initial absorbance
A1 = the absorbance after decolorization

Laccase activity assay

The reaction solution for laccase activity was measured using a TECAN infinite 200 pro microplate reader (Switzerland) at 420 nm for 60 s. The reaction mixture contained 200 µL of acetate buffer 0.1 M (pH 4.5), 250 µL of ABTS 2 mM and 50 µL of culture filtrate. Laccase activity (UmL^{-1}) was calculated according to Eq. (3) with molar absorptivity ($\mathcal{E}$) of 36,000 $M^{-1}cm^{-1}$ [18].

$$\text{Laccase activity } (\text{UmL}^{-1}) = \frac{(\text{Abs.(t)} - \text{Abs.(0)} \times \text{Vtotal mixture (mL)} \times 10^3}{\text{ε} \times \text{V.enzyme (mL)} \times \text{t} \times \text{d}} \quad (3)$$

Abs. (0) = the initial absorbance
Abs. (t) = the final absorbance
10^3 = correction factor (µmol/mol)
t = reaction time (1 min)
d = length of the cell

Manganese peroxidase activity assay

The reaction mixture of manganese peroxidase (MnP) activity was measured using a TECAN infinite 200 pro microplate reader (Switzerland) at 470 nm for 60 s. The reaction mixture contained 100 µL culture filtrate, 175 µL malonic buffer 50 mM (pH 4.5), 12.5 µL 2,6 DMP 20 mM, 12.5 µL $MnSO_4$ 20 mM, 30 µL H_2O_2 2 mM. MnP activity (UmL^{-1}) was calculated according to Eq. (4) [9].

$$\text{MnP activity } (\text{UmL}^{-1}) = \frac{(\text{Abs.(t)} - \text{Abs(0)} \times \text{Vtotal mixture (mL)} \times 10^3}{\varepsilon \times \text{V.enzyme (mL)} \times \text{t} \times \text{d}} \tag{4}$$

Abs. (0) = the initial absorbance
Abs. (t) = the final absorbance
10^3 = correction factor (µmol/mol)
(ε) = 46,600 $M^{-1}cm^{-1}$
t = reaction time (1 min)
d = length of the cell

Results and discussion

Screening of isolates on solid medium

Initial screening used the plate method with a qualitative approach (**Figure 1**). Five isolates showed the ability to decolorize a mixture of commercial direct dyes (DY, DT and DO), which was reflected in the change in the color of the media around the isolates. The reaction between the screening indicator compound and the laccase enzyme secreted by the fungus reflects the color change around the fungus [20,21].

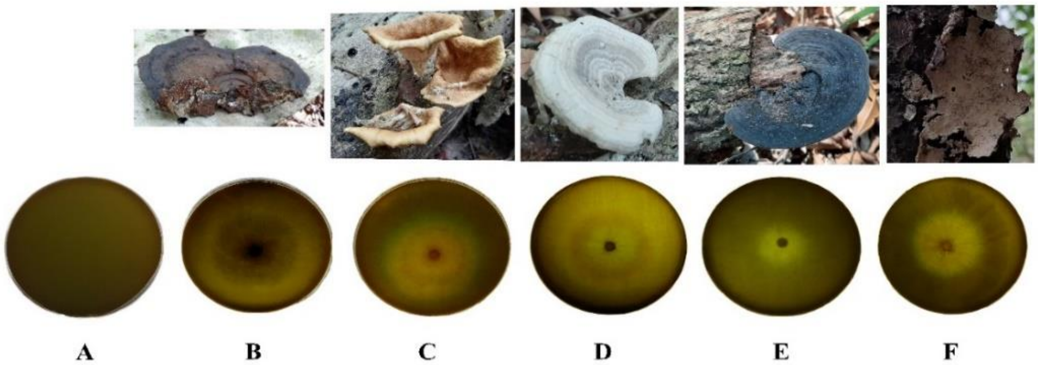

Figure 1 The screening of fungi on a solid medium containing 500 mgL^{-1} mixture of commercial direct dyes containing (A) Control, (B) *Phellinus noxius* (BRB 11), (C) *Lentinus sajor-caju* (BRB 12), (D) *Leotrametes menziesii* (BRB 73), (E) *Flavodon flavus* (BRB 74), and (F) *Ceriporia lacerate* (BRB 81).

Decolorization mixture of commercial direct dyes by WRF isolates

Figure 2 illustrates the decolorization capacity of the WRF isolates when cultivated in liquid media. It has been shown that all fungal isolates possess the capacity to decolorize a mixture of commercially available direct dyes. The decolorization of BRB 12, BRB 11, BRB 73, BRB 74 and BRB 81, which are direct dye mixtures, occurred at rates of 62, 62, 57, 50 and 33 %, respectively, after 96 h. Nevertheless, it is worth noting that 3 isolates, specifically BRB 11, BRB 12 and BRB 73, exhibited laccase and MnP activity while undergoing dye decolorization, as depicted in **Figures 1(C) - 1(D)**. The experimental results indicate that BRB 73 exhibited the most significant enzyme activity, specifically laccase and MnP, with values of 193.8 and 18.4 UL^{-1}, respectively.

The presence of both enzymes in the decolorization indicated that the 3 isolates removed dyes by degradation mechanism. Both enzymes play a role in the mechanism of dye degradation [9,22]. Decolorization mechanisms involving enzymes are known as biodegradation [23]. This mechanism often occurs in fungi because it can produce several non-specific extracellular and intracellular enzymes involved in the process of removing dyes. Research results by Ekanayake and Manage [24] stated that extracellular enzymes were significantly more involved in the decolorization of CI Direct Blue 201.

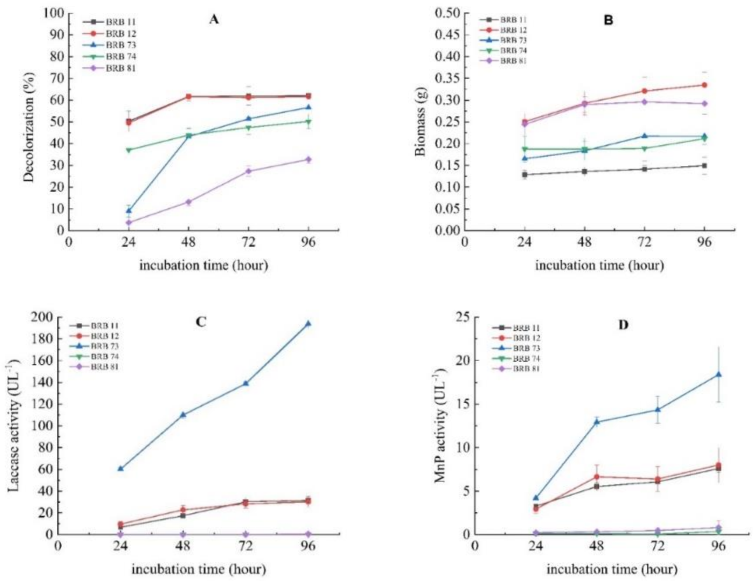

Figure 2 The characterization of WRF isolates in liquid medium containing a mixture of commercial direct dyes in (A) decolorization (%), (B) biomass (g), (C) Laccase activity (UL^{-1}), and (D) MnP activity (UL^{-1}).

In addition, BRB 74 and BRB 81 have low enzyme activities, and no increase in biomass but decolorization can reach 50 and 33 %, respectively. This indicates that the mechanism of decolorization in living cells becomes complex when it involves living organisms. Especially fungi, which produce various enzymes for their metabolism. It becomes difficult to know which specific enzymes play a role in dye degradation. So, it is possible that other enzymes are involved. As reported by Ekanayake and Manage [24] that *A. niger* can produce ligninolytic enzymes, namely LP, MnP, tyrosine, laccase and azoreductase during the textile dye decolorization process. Another possible mechanism that could occur is biosorption of fungal mycelium [23]. This mechanism occurs in either living or dead biomass [25]. Dye molecules can bind to cell wall constituents, such as polysaccharides, proteins, lipids and some groups such as amino ($-NH_2$), carboxyl (-COOH), thiol (-SH), phosphate (PO_4^{3-}) and hydroxyl (-OH). Other fungal cell wall constituents such as chitin and chitosan, elements of the microfibrillar layer structures of fungal cell walls, play an important role in this mechanism as well [26]. In fact, there was no increase in biomass until the end of the observations, so a second mechanism could be possible.

Decolorization single commercial direct dye by selected isolates

Three fungal isolates, BRB 11, BRB 12 and BRB 73 were examined for their capacity to decolorize a single dye. The fungi demonstrated varied proficiency in decolorizing individual dyes, as depicted in **Figures 3 - 5**. All 3 isolates showed decolorization capabilities for DT. The decolorization percentages achieved by the isolates BRB 11, BRB 12 and BRB 73 were, respectively measured to be 46.4, 56.6 and 61.6 % over a span of 96 h. Laccase activities were 231, 19.2 and 551 UmL^{-1}, respectively. MnP activities were 11.4, 2.7 and 32.9 UmL^{-1}, respectively. BRB 11 showed faster decolorization than other isolates. Decolorization within 24 h showed 41.5 %, but there was no significant increase after 96 h of incubation.

According to **Figure 4**, it can be seen that single commercial direct dyes DO exhibit significant resistance to decolorization. BRB 73 achieved the maximum decolorization percentage of 47.8 %. BRB 11 exhibited the lowest decolorization percentage of 1.8 % during a 96-hours timeframe. However, laccase and MnP enzyme activities were detected from the beginning to the end of incubation. Enzyme activities was highest at 96 h of incubation, 188 and 27 UmL^{-1}, respectively. The decolorization process facilitated by BRB 73 was substantiated by the existence of laccase and MnP, with respective concentrations of 207.8 and 14.1 mL^{-1}.

According to **Figure 5**, it can be seen that the decolorization process is more efficient for the single commercial direct dye DY as compared to DT and DO. The decolorization percentage achieved a value of 64.8 % during 24 h period using BRB 11. However, there was no further rise seen until the 96 h incubation period. In the span of 96 h, the decolorization percentages for BRB 12 and BRB 73 were recorded as 66.5 and 67.5 %, respectively. Laccase activities were 176.4, 13.5 and 293 UmL^{-1}, respectively. MnP activities were 7.13, 2 and 25 UmL^{-1}, respectively.

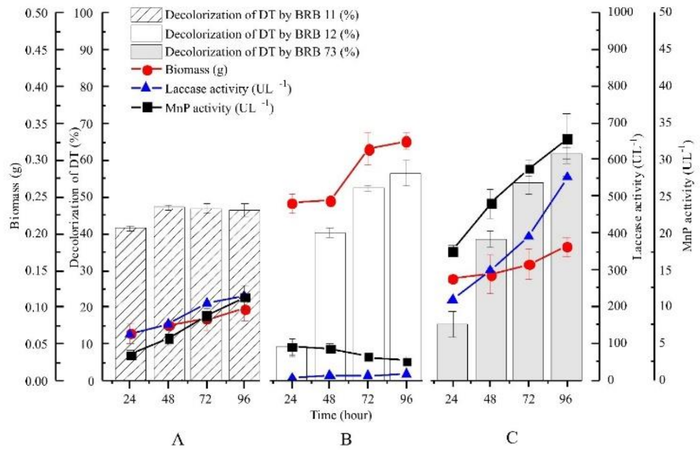

Figure 3 The decolorization of DT and enzyme activities in a liquid medium containing 100 mgL^{-1} by fungi isolates of (A) BRB 11, (B) BRB 12, and (C) BRB 73.

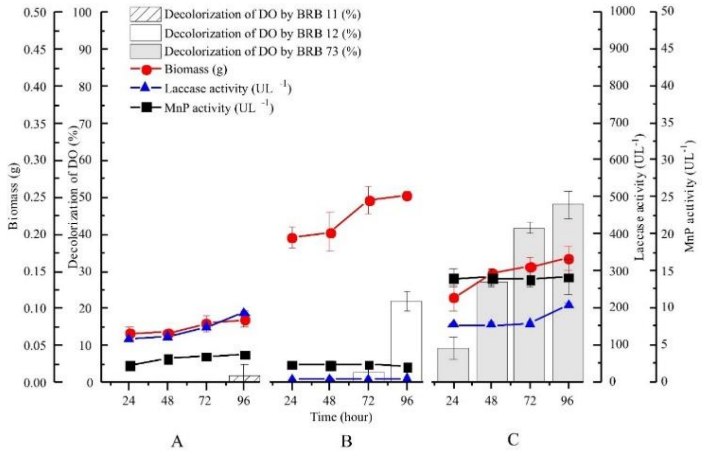

Figure 4 The decolorization of DO and enzyme activities in a liquid medium containing 100 mgL^{-1} by fungi isolates of (A) BRB 11, (B) BRB 12, and (C) BRB 73.

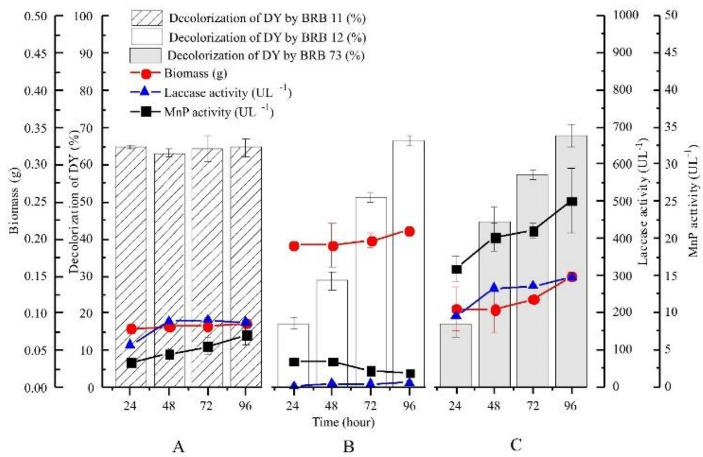

Figure 5 The decolorization of DY and enzyme activities in a liquid medium containing 100 mgL^{-1} by fungi isolates of (A) BRB 11, (B) BRB 12, and (C) BRB 73.

BRB 11 and 12 (**Figure 2**) showed a faster response in decolorization of mixture direct dyes than BRB 73. Especially BRB 11, which decolorizes DT and DY within 24 h (**Figures 3** and **5**). This isolate has the potential to be decolorized in a short time. However, the results of decolorization showed no significant increase until 96 h of incubation. This condition indicates that it has reached the stationary phase, where decolorization becomes stable [27]. This phase occurs in the batch fermentation process due to the limitation of nutrients such as glucose, ammonium and phosphate [28].

A notable increase in biomass and enzyme activities was observed for isolate BRB 73 during this period, while no such change was noticed for the other 2 isolates (**Figures 3** and **5**). This fact illustrates that the 3 WRF have different ways of decolorization. single direct dyes. Decolorization percentage is similar to the temporal profile with an increase in both the activity of laccase and MnP enzymes and biomass by BRB 73 which indicates that both enzymes play a major role in the degradation process. The same temporal profile between decolorization and enzyme activity is similar to that occurring in *Trametes versicolor* [29].

The results showed that lactase was more prominent and played a major role in the decolorization of dyes by BRB 73. Laccase degrades azo dyes in 2 steps. In the first step, azo dyes are degraded to phenolic compounds by nonspecific free radical mechanism [30]. These free radicals are formed any time; 1 electron is removed or added to the ground state of a chemical. Such free radicals are very reactive and rapidly give up or abstract an electron from another chemical [14]. In the next step, the phenolic compounds formed are oxidized to carbonium ions either by nucleophilic attack or by phenoxy radicals generated during the reaction [30]. Laccase produced by fungi can decolorize azo dyes such as Orange G, Congo Red, Direct Blue 15, Rose Bengal and Direct Yellow 27 [31].

In contrast to BRB 11 and 12 which indicate that these 2 enzymes are not major actors in the degradation of these dyes because both decolorization and enzymes are not correlated (**Figures 3** and **6**). It was found in *Pleurotus pulmonarius* [29] and isolates isolated from peatland [32].

The decolorization of DT and DY dyes by the 3 chosen fungi is comparatively more efficient than that of DO dyes. The decolorization capacity of fungus is contingent upon the chemical composition of the dye. Direct dyes are a specific category of azo dyes [2]. Azo compounds that include sulfo, methyl, methoxy or nitro groups exhibit greater resistance to degradation compared to those having amino or hydroxyl groups. According to the Anita *et al.* [9], it has been observed that monoazo groups exhibit a higher rate of decolorization compared to diazo and triazo groups. Moreover, it is well acknowledged that the presence of an azo link, together with an electron-dense area and a sulfonated group in reactive dyes, confers a high degree of recalcitrance. Consequently, the degradation of such dyes by pure cultures is sometimes challenging [30]. Anita *et al.* [9] reported that the decolorization percentages for mono azo (Acid Orange 7), diazo (Reactive Black 5) and trimethyl methane (Methyl Violet) dyes were 13.99 ± 0.30, 7.61 ± 0.01 and 7.59 ± 0.18 %, respectively.

Characterization of selected isolates in liquid medium containing a mixture of commercial direct dyes at various temperature

Understanding the appropriate temperature range for the fungus is of paramount significance as it directly impacts both the development rate and the efficacy of the fungus in the process of dye decolorization. **Figure 6** illustrates that the optimal temperature range for the 3 chosen isolates varied between 30 - 35 °C. The temperature at which the greatest increase in biomass was seen among the 3 fungi was 30 °C. However, it was determined that the most favorable temperature for the decolorization of dyes and the activity of enzymes using BRB 11 and BRB 73 was found to be 35 °C.

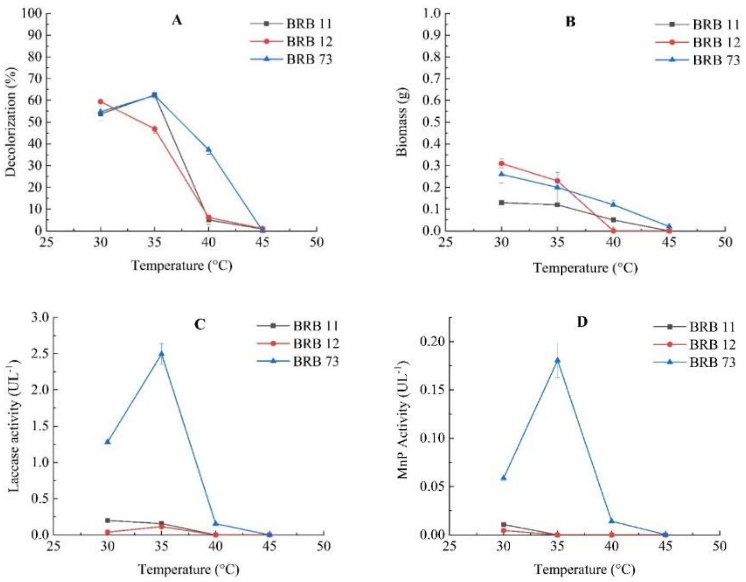

Figure 6 The characterization of 3 isolates (BRB 11, BRB 12 and BRB 73) in a liquid medium containing 100 mgL^{-1} mixture of commercial direct dyes with parameters of (A) decolorization (%), (B) biomass (g), (C) Laccase activity (UL^{-1}), and (D) MnP activity (UL^{-1}).

The decolorization percentages of a combination of commercial direct dyes by BRB 11, BRB 12 and BRB 73 at a temperature of 35 °C were found to be 61.4, 47.4 and 60.7 %, respectively. Fungi reported to have optimum decolorization temperatures ranging from 30 - 35 °C, including Podoscypha elegans (G. Mey) at 30 °C [31], Aspergillus XJ-2 at 35 °C [33], Arthrographis kalrae at 35 °C [34]. BRB 73 can decolorize dyes up to 38 % at 40 °C, which indicates that this isolate is more tolerant to high temperatures than other isolates. Metabolic processes are affected by temperature, including nutrient uptake [35]. High temperatures can cause a decrease in cell viability and denaturation of ligninolytic enzymes [36]. Optimal temperature is needed to optimize the decolorization of dyes by enzymes produced by organisms [35].

Toxicity assay

A toxicity assay was carried out using *Artemia salina* as a model. This small aquatic organism can be used as an efficient bioindicator to predict dye toxicity because it is sensitive to environmental changes [37]. The findings are shown in **Table 1**. These crustaceans are often used as a toxicity model for the assessment of different contaminants, such as dyes. In some instances, the metabolites generated from the breakdown of the dye by laccases exhibit higher toxicity levels compared to the original compound [12]. Hence, it is important to ascertain that the decolorization process facilitated by WRF adheres to ecologically sustainable practices.

Table 1 shows that the cultures subjected to WRF treatment exhibited decreased levels of toxicity in comparison to untreated cultures. Indications of a detoxification process were found that the LC_{50} value of *Artemia salina* treated was higher than untreated, either single or mixture of dyes. Dyes that enter the body of *Artemia* sp. accumulated in the mid-gut region, which cause increased mortality when the dye concentration increased [38].

The treatment by BRB 11 and BRB 73 gave a good response to reduce the toxicity level of mixed commercial direct dyes. However, the reduction in toxicity levels of single dyes (turquoise, yellow and orange) was highest by BRB 11 (**Table 1**). Other studies report that WRF can decolorize and detoxify synthetic dyes such as *Lentinus arcularius* [39], *Aspergillus niger* and *Trichoderma viride* [40].

Table 1 Toxicity assay of commercial direct dye mixture and a single direct dye by 3 selected isolates.

Dyes	Samples	Regression equation	R^2	LC_{50} (mgL^{-1})
Mixture direct dyes	Untreated (control)	y = 9.2889x – 6.522	0.8527	15.37 ± 1.9
		y = 7.9214x – 4.3174	0.9476	
		y = 4.2454x + 0.172	0.9269	
	BRB 11	y = 2.779x + 0.5988	0.9809	35.84 ± 2.2
		y = 3.0604x + 0.2905	0.9781	
		y = 3.0604x + 0.2905	0.9781	
	BRB 12	y = 9.9578x – 4.4229	0.9408	21.63 ± 1.4
		y = 4.0881x – 0.3226	0.9096	
		y = 7.0746x – 4.528	0.9466	
	BRB 73	y = 12.143x – 14.333	0.9259	34.36 ± 4.4
		y = 5.8992x – 4.0074	0.9869	
		y = 10.196x – 10.111	0.9789	
Turquoise direct dye	Untreated (control)	y = 8.5186x – 2.9374	0.9472	8.55 ± 0
		y = 8.5186x – 2.9374	0.9472	
	BRB 11	y = 1.7951x + 1.8591	0.9259	56.64 ± 0.6
		y = 1.8174x + 1.8077	0.9667	
	BRB 12	y = 3.93x + 0.0652	0.9393	18.5 ± 0.7
		y = 4.0579x – 0.1874	0.9458	
	BRB 73	y = 2.4997x + 2.0439	0.9555	14.98 ± 0.35
		y = 4.9213x – 0.7494	0.971	
Yellow direct dye	Untreated (control)	y = 2.9411x + 1.6336	0.9158	13.86 ± 0.1
		y = 2,9336x + 1.6597	0.9261	
	BRB 11	y = 1.3102x + 2.52	0.9625	75.73 ± 3.4
		y = 1.3253x + 2.528	0.9635	
	BRB 12	y = 2.1101x + 1.8895	0.9393	31.75 ± 2.8
		y = 1.7687x + 2.2978	0.9723	
	BRB 73	y = 6.2786x – 2.6657	0.9716	17.15 ± 0.74
		y = 6.1483x – 2.6692	0.9853	
Orange direct dye	Untreated (control)	y = 6.8459x – 1.5586	0.9308	9.23 ± 0.2
		y = 6.9911x – 1.7976	0.9314	
	BRB 11	y = 1.3102x + 2.52	0.9948	142.7 ± 0
		y = 1.2814x + 2.2394	0.9948	
	BRB 12	y = 2.8908x + 1.6746	0.9857	13.37 ± 1.1
		y = 2.8466x + 1.868	0.9973	
	BRB 73	y = 4.4648x – 0.6703	0.9769	18.41 ± 0.31
		y = 4.7652x – 1.0033	0.977	

Conclusions

This research suggests the promising potential of 3 fungal isolates specifically, *Phellinus noxius* (BRB 11), *Lentinus sajor-caju* (BRB 12) and *Leotrametes menziesii* (BRB 73) sourced from TNBS in Indonesia. *Phellinus noxius* (BRB 11) can decolorize commercial direct dyes, both mixed dyes and single dyes in a shorter time than other isolates. However, this isolate entered the stationary phase more quickly than the other isolates, indicating faster nutrient consumption. *Leotrametes menziesii* (BRB 73) indicated that the dye decolorization process was through a degradation pathway involving enzymes, both laccase and MnP. The optimal temperature for *Phellinus noxius* (BRB 11) and *Leotrametes menziesii* (BRB 73) is 35 °C, while *Lentinus sajor-caju* (BRB 12) is 30 °C. The third isolate demonstrated that it could reduce the toxicity of dyes, both single and mixed dyes. However, the lowest toxicity was by *Phellinus noxius* BRB 11. This study proposes the potential of 2 isolates: *Phellinus noxius* (BRB 11) and *Leotrametes menziesii* BRB 73, from TNBS for degradation of single and mixture dye wastewater in the environment.

Acknowledgments

The authors are grateful to National Research and Innovation Agency (BRIN), Indonesia for providing financial support and research facilities. The authors also thank the facilities and technical assistance of the Integrated Laboratory of Bioproducts (ILaB) at the National Research and Innovation Agency (BRIN), Indonesia.

References

[1] N Sekar. *12 - Direct dyes*. Handbook of Textile and Industrial Dyeing. Woodhead Publishing, Sawston, 2011, p. 425-45.
[2] S Chaturvedi. Azo dyes decolorization using white rot fungi. *Res. Rev. J. Microbiol. Biotechnol.* 2019; **8**, 9-19.
[3] J Sharma, S Sharma and V Soni. Classification and impact of synthetic textile dyes on Aquatic Flora: A review. *Reg. Stud. Mar. Sci.* 2021; **45**, 101802.
[4] T Sheela and SK Sadasivam. Dye degradation potential and its degradative enzymes synthesis of *Bacillus cereus* skb12 isolated from a textile industrial effluen. *J. Appl. Biol. Biotechnol.* 2020; **8**, 42-6.
[5] OD Nurhayat. Potency of Indonesian white-rot fungus as biodecolorization agent for synthetic dye wastewaters. *Jurnal Mikologi Indonesia* 2020; **4**, 156-67.
[6] M Biruntha, J Archana, K Vanimuthu, BK Selvi, JAJ Paul, RM Vithyavathy, K Kaviyarasu and G Ramalingan. Efficacy of dye degradation of contaminated soil microbial isolates. *Mater. Today Proc.* 2021; **36**, 167-70.
[7] TT Bogale. Biotechnological applications of white rot fungi: A review. *GSC Adv. Res. Rev.* 2020; **5**, 97-103.
[8] OD Nurhayat, FC Ardiati, KP Ramadhan, SH Anita, H Okano, T Watanabe and DHY Yanto. Bioprospecting three newly isolated white-rot fungi from Berbak-Sembilang National Park, Indonesia for biodecolorization of anthraquinone and azo dyes. *Biodiversitas J. Biol. Divers.* 2022; **23**, 613-23.
[9] SH Anita, FP Sari and DHY Yanto. Decolorization of synthetic dyes by ligninolytic enzymes from *Trametes hirsuta* D7. *Makara J. Sci.* 2019; **23**, 44-50.
[10] DHY Yanto, N Auliana, SH Anita and T Watanabe. Decolorization of synthetic textile dyes by laccase from newly isolated *Trametes hirsuta* EDN084 mediated by violuric acid. *IOP Conf. Ser. Earth Environ. Sci.* 2019; **374**, 012005.
[11] A Hidayat and S Tachibana. Decolorization of azo dyes and mineralization of phenanthrene by *Trametes* sp. AS03 isolated from Indonesian Mangrove Forest. *Indonesian J. Forest. Res.* 2014; **1**, 67-75.
[12] SK Sen, S Raut, P Bandyopadhyay and S Raut. Fungal decolouration and degradation of azo dyes: A review. *Fungal Biol. Rev.* 2016; **30**, 112-33.
[13] RL Singh, PK Singh and RP Singh. Enzymatic decolorization and degradation of azo dyes - a review. *Int. Biodeterioration Biodegradation* 2015; **104**, 21-31.
[14] L Singh. Biodegradation of synthetic dyes: A mycoremediation approach for degradation/ decolourization of textile dyes and effluents. *J. Appl. Biotechnol. Bioeng.* 2017; **3**, 430-5.
[15] S Huang, S Li, Z Wang, S Lin and J Deng. Enzyme degradation mechanism of white rot fungi and its research progress on Refractory Wastewater. *E3S Web Conf.* 2021; **237**, 01002.
[16] APD Almeida, A Macrae, BD Ribeiro and RPD Nascimento. Decolorization and detoxification of

different azo dyes by *Phanerochaete chrysosporium* ME-446 under submerged fermentation. *Braz. J. Microbiol.* 2021; **52**, 727-38.
[17] S Reddy and WJ Osborne. Heavy metal determination and aquatic toxicity evaluation of textile dyes and effluents using *Artemia salina*. *Biocatalysis Agr. Biotechnol.* 2020; **25**, 101574.
[18] R Alam, FC Ardiati, NN Solihat, B Alam, SH Lee, DHY Yanto, T Watanabe and S Kim. Biodegradation and metabolic pathway of anthraquinone dyes by *Trametes hirsuta* D7 immobilized in light expanded clay aggregate and cytotoxicity assessment. *J. Hazard. Mater.* 2021; **405**, 124176.
[19] SH Anita, FC Ardiati, M Oktaviani, FP Sari, OD Nurhayat, KP Ramadhan and DHY Yanto. Immobilization of laccase from *Trametes hirsuta* EDN 082 in light expanded clay aggregate for decolorization of Remazol Brilliant Blue R dye. *Bioresource Tech. Rep.* 2020; **12**, 100602.
[20] KO Omeje, NE Nnolim, BO Ezema, JN Ozioko and SOO Eze. Synthetic dyes decolorization potential of agroindustrial waste-derived thermo-active laccase from *Aspergillus* species. *Biocatalysis Agr. Biotechnol.* 2020; **29**, 101800.
[21] AT Thakkar and SA Bhatt. Isolation, identification and optimization of laccase from *Alternaria alternata*. *J. Appl. Biol. Biotechnol.* 2020; **8**, 64-9.
[22] C Ning, L Qingyun, T Aixing, S Wei and L Youyan. Decolorization of a variety of dyes by *Aspergillus flavus* A5p1. *Bioproc. Biosystems Eng.* 2018; **41**, 511-8
[23] B Dayl, C Onac, A Kaya, HA Akdogan and S Rodriguez-Couto. New type biomembrane: Transport and biodegradation of reactive textile dye. *ACS Omega* 2020; **5**, 9813-9.
[24] MS Ekanayake and P Manage. Mycoremediation potential of synthetic textile dyes by *Aspergillus niger* via biosorption and enzymatic degradation. *Environ. Nat. Resour. J.* 2022; **20**, 234-45.
[25] P Yang, W Shi, H Wang and H Liu. Screening of freshwater fungi for decolorizing multiple synthetic dyes. *Braz. J. Microbiol.* 2016; **47**, 828-34.
[26] T Lu, QL Zhang and SJ Yao. *Application of biosorption and biodegradation functions of fungi in wastewater and sludge treatment. In*: D Purchase (Ed.). Fungal applications in sustainable environmental biotechnology. Springer, Switzerland, 2016, p. 65-90.
[27] AM Restrepo, FM Otalvaro, CV Quintero and AH Anaguano. Evaluation of several white-rot fungi for the decolorization of a binary mixture of anionic dyes and characterization of the residual biomass as potential organic soil amendment. *J. Environ. Manag.* 2020; **254**, 109805.
[28] P Vrabl, CW Schinagl, DJ Artmann, B Heiss and W Burgstaller. Fungal growth in batch culture - what we could benefit if we start looking closer. *Front. Microbiol.* 2019; **10**, 2391.
[29] S Jiménez, C Velásquez, F Mejía, M Arias and A Hormaza. Comparative studies of pure cultures and a consortium of white-rot fungi to degrade a binary mixture of dyes by solid-state fermentation and performance at different scales. *Int. Biodeterioration Biodegradation* 2019; **145**, 104772.
[30] A Mani and SAS Hameed. Improved bacterial-fungal consortium as an alternative approach for enhanced decolourisation and degradation of azo dyes: A review. *Nat. Environ. Pollut. Tech.* 2019; **18**, 49-64.
[31] S Pramanik and S Chaudhuri. Laccase activity and azo dye decolorization potential of *Podoscypha elegans*. *Mycobiology* 2018; **46**, 79-83.
[32] E Noman, BA Talip, A Al-Gheethi, R Mohamed and H Nagao. Decolourisation of dyes in greywater by mycoremediation and mycosorption process of fungi from peatland; primary study. *Mater. Today Proc.* 2020; **31**, 23-30.
[33] Y Ge, B Wei, S Wang, Z Guo and X Xu. Optimization of anthraquinone dyes decolorization conditions with response surface methodology by *Aspergillus*. *Kor. Chem. Eng. Res.* 2015; **53**, 327-32.
[34] A Yadav, P Yadav, AK Singh, V Kumar, VC Sonawane, Markandeya, RN Bharagava and A Raj. Decolourisation of textile dye by laccase: Process evaluation and assessment of its degradation bioproducts. *Bioresource Tech.* 2021; **340**, 125591.
[35] R Khan and MH Fulekar. Mineralization of a sulfonated textile dye Reactive Red 31 from simulated wastewater using pellets of *Aspergillus bombycis*. *Bioresource Bioprocessing* 2017; **4**, 23.
[36] MA Hefnawy, MM Gharieb, MT Shaaban and AM Soliman. Optimization of culture condition for enhanced decolorization of direct blue dye by *Aspergillus flavus* and *Penicillium canescens*. *J. Appl. Pharmaceut. Sci.* 2017; **7**, 83-92.
[37] D Rawat, V Mishra and RS Sharma. Detoxification of azo dyes in the context of environmental processes. *Chemosphere* 2016; **155**, 591-605.
[38] SS Babu, C Mohandass, AS Vijayaraj and MA Dhale. Detoxification and color removal of Congo red by a novel *Dietzia* sp. (DTS26) - a microcosm approach. *Ecotoxicol. Environ. Saf.* 2015; **114**, 52-60.
[39] C Bayburt, AB Karaduman, BY Gürsu, M Tuncel and M Yamaç. Decolourization and detoxification

of textile dyes by *Lentinus arcularius* in immersion bioreactor scale. *Int. J. Environ. Sci. Tech.* 2020; **17**, 945-58.
[40] E Ali, I Amjad and A Rehman. Evaluation of azo dyes degradation potential of fungal strains and their role in wastewater treatment. *Saudi J. Biol. Sci.* 2023; **30**, 103734.