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Application of ligninolytic enzyme of *Lentinus polychrous* on synthetic dye decolorization

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Abstract. Contamination of water resources by synthetic dyes causes many environmental and health problems. Fungal ligninolytic enzymes have been applied for dye decolorization due to its ability in degrading a wide variety of recalcitrant substances. Extracellular ligninolytic enzyme production by white rot fungus, *Lentinus polychrous*, grown in glucose containing medium supplemented with rice straw powder and soybean pomace was monitored. Optimum condition for remazol brilliant blue R (RBBR) decolorization was studied by varying initial RBBR concentrations, pH values, and initial crude enzyme concentrations. The result revealed that the ligninolytic enzyme dominantly produced was laccase with an activity of 0.095 U/ml on day 15, and a small amount of manganese peroxidase was also detected. The crude laccase produced from *L. polychrous*, effectively decolorized the dye within a short period of time, that was approximately 50% of 20 mg/l RBBR was decolorized within the first 2 hours. The optimal pH for RBBR decolorization was 3.0 which had an efficiency of 87% within 6 hours after incubation. Moreover, the best redox mediator of laccase was $CuSO_4$, whose decolorization efficiency was over twice than that of samples without this mediator. The decolorization intensified with increase of $CuSO_4$ concentration but higher concentrations of chromium tended to suppress decolorization.

1. Introduction

Synthetic dyes have been widely used in textile, paper, cosmetic, food, and pharmaceutical industries. Textile industry consumes large amounts of water supply and produces tremendous amounts of highly colored effluent containing up to 30% of unfixed dyes and other organic compounds, that is released during the dyeing process [1]. Based on different structures in chromophores of the synthetic dyes, they are classified into several groups, like azo, anthraquinone, indigo, triphenylmethyl and phthalocyanine dyes, containing complex aromatic and heterocyclic structures. These complex structures make the dyes toxic, impermeable to light, and barely biodegradable when discharged into the environment [2]. Furthermore, the dyes hold carcinogenic and mutagenic properties which multiple adverse effects on the environment and health. Dye-containing effluent commonly has high biological oxygen demand (BOD), chemical oxygen demand (COD), and high salt concentrations [1]. The dye-containing effluent accumulates in the water body obstructs sunlight penetrating through the water, thereby reducing photosynthesis of aquatic plants and resulting in lower oxygen concentration in the water. Moreover, as microbes also require dissolved oxygen to biologically degrade excess amounts of contaminated dyes, these dyes in the water, eventually cause oxygen depletion, with negative environmental consequences.

Biological decolorization is a promising approach due to its advantages like being an environmentally friendly process, lower chemical and energy consumptions, and reduced cost in breakdown of organic compounds [3]. Several studies have demonstrated that fungi are able to degrade complex organic substances using extracellular ligninolytic enzymes like laccase, manganese peroxidase, and lignin peroxidase [4]. Ability of the fungi to produce each ligninolytic enzyme is different among fungal species due to differences in genetic materials and culture conditions. Some fungi can produce laccase and manganese peroxidase, while others can produce only laccase [5, 6]. In general, application of fungi or their extracellular enzymes are more favorable than those of other organisms since fungi can decolorize several types of synthetic dyes having different chemical structures [6], and ligninolytic enzymes originating from different fungal species can breakdown the same dye at different levels [7]. Therefore, investigation of organisms useful in degrading several complex and recalcitrant pollutants is an important approach in bioremediation.

Laccase is a copper-containing oxidoreductase enzyme recognized for its broad specificity in catalyzing a wide range of substrates like lignin, phenolic and non-phenolic molecules, synthetic dyes and aromatic amines coupled with reduction of oxygen [8]. It is evident that laccase production can be enhanced by addition of aromatic inducers like benzyl alcohol, guaiacol, veratryl alcohol, and some heavy metals [3, 9]. Laccase from several fungal species has been studied for its ability in decolorization. Most of them are *Trametes* sp., *Phanerochaete* sp., *Funalia* sp., *Pleurotus* sp. [10] such as *Trametes versicolor*, *Trametes trogii*, *Phanerochaete chrysosporium*, *Aspergillus* sp., *Pleurotus ostreatus*, *Coriolus versicolor* and *Funalia trogii* [5, 8, 11-13]. In this study, a member of the relatively under-investigated *Lentinus* sp., which is *Lentinus polychrous*, was studied for its decolorizing performances using extracellular ligninolytic enzymes. *L. polychrous* is a famous edible wood-degrading white rot fungus commonly found in northern and northeastern regions of Thailand. This research aimed to monitor ligninolytic enzyme activity produced from *L. polychrous* and apply the crude enzyme for decolorization of remazol brilliant blue R (RBBR). RBBR is a sulfonated anthraquinone dye used as a starting substance for polymeric dye in textile industry [14] and considered as a recalcitrant organopollutant containing complex aromatic structure. In this study, several parameters like pH values, initial dye concentrations and initial enzyme concentrations, were varied to determine the optimal condition for RBBR decolorization, as well as effects of mediators and heavy metal ions on decolorization.

2. Materials and methods

2.1. Materials

RBBR (reactive blue 19), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and hydroxybenzotriazole hydrate (HBT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bromophenol blue was obtained from LabChem. Methyl orange, chromium (III) chloride, and copper (II) sulphate were obtained from M&B, QReC, and Univar, respectively.

2.2. Culture condition for ligninolytic enzyme production

The fungus, *L. polychrous*, was grown on freshly prepared potato dextrose agar (PDA) for one week to obtain the active fungal mycelium. Then, three mycelial bits (7 mm each) were transferred to 250 ml Erlenmeyer flasks containing 100 ml potato dextrose broth (PDB) supplemented with 0.1 g of rice straw powder and soybean pomace. The cultures were incubated at 27°C, 110 rpm for ligninolytic enzyme production and enzyme activity in the supernatant was monitored every three days until day 15. Thereafter, the culture was centrifuged at 12,000 rpm for 10 min to obtain the mycelial free crude enzyme, which would subsequently be tested for dye decolorization.

2.3. Ligninolytic enzyme activity determination

Enzyme activity of the three main ligninolytic enzymes, which are laccase, manganese peroxidase, and lignin peroxidase were monitored every three days. Laccase activity was determined using 2,2'-azino-

bis (3-ethylbenzothiazoline-6-sulphonic acid, ABTS) in acetate buffer, pH 4.5. Oxidation of ABTS was spectrophotometrically determined by an increase in absorbance at 420 nm ($\varepsilon_{420} = 0.036\ \mu M^{-1}cm^{-1}$) [4]. Measurement of manganese peroxidase and lignin peroxidase was modified from Bholay *et al.*, 2012. Manganese peroxidase was monitored using 0.1 mg/ml phenol red in the presence of 0.1 mM manganese sulfate, 1 mg/ml of bovine serum albumin, 0.1 mM hydrogen peroxide, 25 mM sodium lactate, and 0.5 ml culture filtrate [15]. Oxidation of phenol red was monitored at 610 nm ($\varepsilon_{610} = 0.0446\ \mu M^{-1}cm^{-1}$) [16]. Lignin peroxidase was determined by monitoring the demethylation of methylene blue to azure C [15]. The reaction contained 32 µM methylene blue in 50 mM sodium tartarate buffer (pH 4.0), 10 µl of culture filtrate and 0.1 ml of 0.1 mM H_2O_2. The reaction was incubated at room temperature for 1 hour and lignin peroxidase activity was measured at A_{650nm}. Percentage of methylene blue decolorization was calculated as (A_{650} of control – A_{650} of test/A_{650} of control) x 100. All enzyme activity reactions were stopped by adding 100 µl of H_2SO_4. One unit of enzyme activity (U) was defined as the amount of enzyme required for oxidation of 1 µmol substrate per minute.

2.4. Effects of different dye concentrations, pH values, and enzyme concentrations on decolorization
Different RBBR concentrations of 20, 40, 60, 80 and 100 mg/l were prepared using citrate phosphate buffer (pH 7.0). Each reaction contained 25 ml of RBBR and 2 ml of crude enzyme. To determine the optimum pH, decolorization of RBBR was performed within the pH ranges of 3.0 – 7.0 and the desired pH of the dye solution was prepared in citrate phosphate buffer. Each reaction contained 25 ml of 20 mg/l dye solution and 2 ml of crude enzyme. To determine the effect of enzyme concentrations on decolorization, crude enzyme amounts of 0.5, 1, 1.5, 2 and 2.5 ml, having laccase activity of 0.095 U/ml, were tested for ability in decolorizing 20 mg/l RBBR. Each reaction contained 25 ml of dye solution and different amounts of enzymes. All reactions were incubated at room temperature without shaking, and decrease in absorbance was spectrophotometrically monitored at 0, 1, 2, 4, and 6 hours at the absorbance of 592 nm. The reactions were tested in triplicate, and the experimental control was the sample without the crude enzyme, performed under the same conditions.

2.5. Effect of mediators and heavy metal on dye decolorization
$CuSO_4$ (10, 20, 30 mg/l) and HBT (1, 5, 10 mM) were used as mediators on RBBR decolorization. $CrCl_4$ (10, 20, 30 mg/l) was used to study effects of heavy metals on RBBR decolorization at the optimum pH. Each mediator and heavy metal was added to 100 mg/l RBBR in citrate phosphate buffer (pH 3.0). Each reaction contained 25 ml of dye solution and 2.5 ml of the crude enzyme. Monitoring of dye decolorization was performed as described above. Absorbance spectra of the untreated and treated samples were scanned using UV-VIS 1800 spectrophotometer (Shimadzu).

2.6. Percentage of dye decolorization
Percentage of dye decolorization was calculated using the following formula.

$$\text{Decolorization efficiency } (\%) = [(A_I - A_T)/ A_I] \times 100$$

where, A_I is initial absorbance of the heat inactivated crude enzyme, A_T is specific time absorbance measured after incubation with the enzyme. The values shown were mean of triplicate with standard deviation.

3. Results and discussion

3.1. Ligninolytic enzyme production
Ligninolytic enzyme production was studied by culturing the active mycelium of *L. polychrous* in PDB medium supplemented with rice straw and soybean pomace. The results revealed that extracellular ligninolytic enzymes secreted by *L. polychrous* were predominantly laccase, along with

low levels of manganese peroxidase. Figure 1 shows that the maximum laccase activity of 0.095 U/ml was obtained on day 15, and the activity was around six folds higher than that of manganese peroxidase. It is possible that laccase activity might increase if longer incubation time is provided, and addition of laccase inducers would stimulate fungal growth and enzyme production. Lignin peroxidase was undetectable under the culture condition, which could be because the fungus lacks genes responsible for lignin peroxidase production or the gene expression is suppressed [17]. For these reasons, laccase was the major enzyme that played a vital role in decolorizing RBBR in this study.

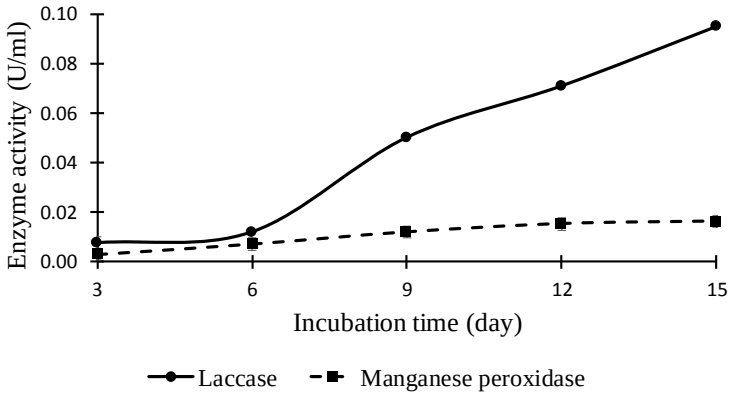

Figure 1. Ligninolytic enzyme production by *L. polychrous*.

Ligninolytic enzyme production shown in the present study was in agreement with Wangpradit *et al.*, 2014 who reported that ligninolytic enzyme produced by *L. polychrous* was only laccase and manganese peroxidase [6]. Another research showed that laccase production of *L. polychrous* on solid substrates, corn husk, rice bran and rice husk was 0.145 U/ml on day 12, which was 1.5 times higher than our study [18]. The different types and compositions of the culture medium as well as culture conditions contribute to different amounts of enzyme production. It has been reported that ligninolytic enzyme production by white-rot fungi substantially depends on medium compositions such as carbon and nitrogen contents [9, 19]. When applying different types of carbon sources, *L. crinitus* grown in 10 g/l glucose decolorized the highest levels of RB220 dye up to 85%, while the culture with glycerol and starch yielded low destaining activity of only 5-10% [19]. This indicated that different types of carbon sources resulted in variable amounts of ligninolytic enzyme production, thus influencing decolorization performance. Apart from *Lentinus* sp., other white rot fungi, *P. ostreatus* strain 32 and *P. ostreatus* HAUCC 162 produced only laccase, while lignin peroxidase and manganese peroxidase were undetectable [8, 20]. They revealed that laccase activity substantially increased with organic nitrogen sources like peptone and yeast extract [8], and several metal ions, especially Cu^{2+}, could promote laccase production [20]. Ligninolytic enzyme of *Polyporus* sp. S133 was mostly laccase, and production of lignin peroxidase and manganese peroxidase was relatively low [21].

3.2. Effect of RBBR concentrations on decolorization

Effect of RBBR concentrations on decolorization efficiency was performed at 20 - 100 mg/l dye concentrations at pH 7.0. After 2 hours, 20 mg/l RBBR was decolorized up to 50%, and more than 40% decolorization was obtained with 40 and 60 mg/l RBBR as shown in Figure 2. It was observed that destaining activity barely increased after 2 hours of incubation, possibly because the enzyme becomes saturated with the substrate or enzyme denaturation occurs during a long incubation period. Decolorization of 100 mg/l dye concentration was only 23% after 6 hours of incubation. The results demonstrated that decolorization efficiency decreased with increasing dye concentrations. Our results are similar to those of Mechichi *et al.*, 2006 and Hadibarata *et al.*, 2011, who revealed that dye

decolorization decreased with increasing concentrations of RBBR, suggesting that the rate of reaction increased with the substrate concentrations until saturation [12, 21]. Also, adding high concentrations of dye as a substrate directly to the culture could be toxic to fungal growth, and the growth of *P. ostreatus* was inhibited when initial RBBR concentration increased to 100 mg/l demonstrating the inhibitory effects of the dye on fungal growth [5].

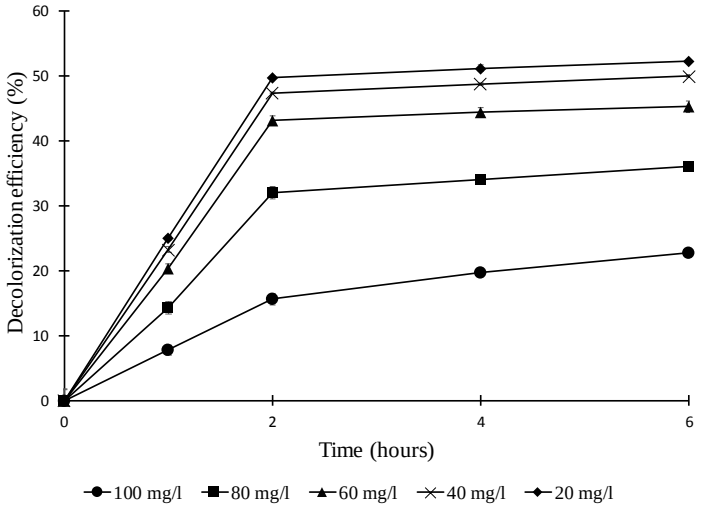

Figure 2. Decolorization of different RBBR concentrations.

3.3. Effect of pH values on decolorization

RBBR decolorization was performed at different pH values from 3.0 to 7.0 in citrate phosphate buffer containing 20 mg/l RBBR and 2 ml of crude enzyme. Among five tested pH values, the maximum decolorization was obtained at pH 3.0 where majority of RBBR decolorization occurred at 78% within 2 hours of incubation and the maximum decolorization of 86% was obtained within 4 hours as shown in Figure 3. It was obvious that the enzyme could effectively decolorize RBBR at low pH ranges from 3.0 to 5.0, and the dye was distinctly degraded within the first 2 hours of incubation, after that the percentage of decolorization barely increased. The decolorization efficiency was greatly decreased at pH 6.0 and 7.0 with a removal efficiency of only 59% and 47% at 6 hours, respectively. It was evident that dye decolorization largely depended on pH levels and the decolorization was more favorable in acidic environment.

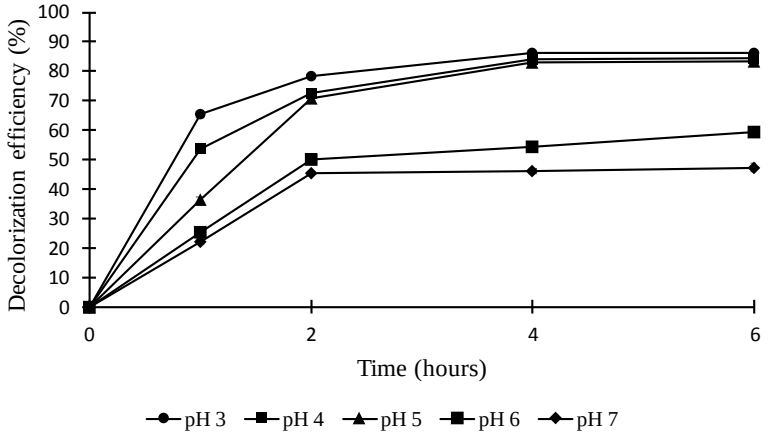

Figure 3. Optimum pH for decolorization of RBBR.

The maximum decolorization efficiency at pH 3.0 was in accordance with Sarnthima *et al*., 2009 who reported that decolorization of 20 mg/l RBBR at pH 3.0 was 58% within 1 hour [22]. They also reported using 0.2 U/ml of crude laccase which was almost twice than that of our study. Another observation was that *L. polychrous* decolorized 66% of RBBR at pH 4.0 within 3.5 hours, whereas the optimum pH on ABTS substrate was 3.0, and relative activity continuously decreased with increasing pH values from 3.0 to 7.0, and the remaining activity of 16% at pH 7.0 [23]. The higher catalytic activity at low pH ranges might contribute to higher performance of RBBR decolorization in acidic environment and small decolorization at neutral pH. It was reported that most laccase produced from fungi have the optimum pH in acidic ranges [14]. Laccase from *Lentinus* sp. showed the optimum pH at 2.5, 3.5, and 5.0 on the substrates ABTS, 2,6-DMP, and guaiacol respectively, indicating that the optimum pH mainly depended on the substrates [14]. It is noticeable that the optimum pH values were distinct in the acidic ranges, where laccase had a higher catalytic performance. Hadibarata *et al*., 2011 found that the optimal RBBR decolorization rate of laccase purified from *Polyporus* sp. S133 was at pH 5.0 and that the crude laccase was unable to decolorize RBBR at pH 7.0 [21].

According to dye's chemical structure and enzyme conformation, electrical charge of the dye and enzyme plays an important role in decolorization. RBBR contains sulfonic ($-SO^{-}_{3}-$) functional group which is negatively charged, while electrical charge at the active site of the enzyme is largely dependent on the pH of the solution. At lower pH values with high concentration of H^{+}, the active site has a strong positively charged facilitating binding with anionic sulfonic group of the RBBR dye; thus, high removal efficiency is achieved at low pH values. On the other hand, an active site at higher pH values contains less positively charged H^{+}, therefore ionic attraction between the dye and enzyme is less, and a reduction in decolorization efficiency is thus observed [24].

To determine the optimum pH of other synthetic dyes, decolorization of bromophenol blue and methyl orange was studied in pH ranges from 3.0 to 7.0 (data not shown). The results showed that both dyes had the optimum pH at 4.0 with maximum decolorization efficiency of 73% and 71% for bromophenol blue and methyl orange, respectively (Table 1). Similar observation was found by Rakrudee 2009 showing that the optimum pH for bromophenol blue decolorization was at pH 4.0 with 37% decolorization efficiency [22]. The dye decolorization in this study indicated that the optimal pH of laccase was substrate dependent and that the enzyme was more active in the acidic range. It was also noteworthy that bromophenol blue and methyl orange decolorization was less than 5% at pH 7.0. However, crude laccase produced from *L. crinitus* was active in a wide range of pH values from acidic to basic conditions and was able to decolorize more than 70% of reactive blue 220 from pH 3.5 to 8.0. [19].

Table 1. Decolorization of other synthetic dyes by *L. polychrous*.

Dyes	Types	Optimum pH	Time (h)	Decolorization (%)
Bromophenol blue	Triphenylmethane	4.0	2 h	70
			12 h	73
Methyl orange	Azo	4.0	2 h	37
			12 h	71

In general, different dye structures affect decolorization capacity and laccase decolorizes anthraquinone dye more effective than other dye groups [18], and the most resistant dyes are triphenylmethane and azo dyes [25]. Our result found that the most degradation-resistant group was azo (methyl orange), followed by triphenylmethane (bromophenol blue), while anthraquinone (RBBR) was the most degradable dye. However, the decolorization efficiency of bromophenol blue was almost as high as that of RBBR, as crude laccase also decolorized bromophenol blue as effectively. Similarly, crude enzyme from *Paraconiothyrium variabile* also had a good capacity in decolorizing the triphenylmethane dye [7]. This could be because different isoforms of crude laccase may be effective

in decolorizing different types of dyes and some isoforms are capable of decolorizing triphenylmethane or azo dyes [20]. Unlike other dyes which were decolorized to reach maximum efficiency within 2 to 4 hours, degradation of methyl orange took 12 hours to reach maximum degradation of 72%, while decolorization at 2 hours was only 32%. This indicated that methyl orange was more resistant than other groups and longer degradation time was required to reach maximum decolorization. It was noteworthy that three types of dyes tested in the present study had optimal pH in acidic ranges, which might contribute to high catalytic performance of the crude laccase in low pH ranges.

3.4. Effect of enzyme concentrations on decolorization

Effect of enzyme concentrations on decolorization varied from 0.5 to 2.5 ml (with laccase activity of 0.095 U/ml). The results revealed that decolorization efficiency increased with increasing enzyme concentrations. The difference in decolorization was obvious within the first two hours after incubation where higher enzyme concentration resulted in higher decolorization rate. The maximum decolorization of 89% was obtained when using 2.5 ml of crude enzyme, and the lowest decolorization of 68% was obtained when using 0.5 ml of crude enzyme as shown in Figure 4. It was noticeable that more than 80% of the dye was decolorized within the first 2 hours when using more than 2 ml of crude enzyme.

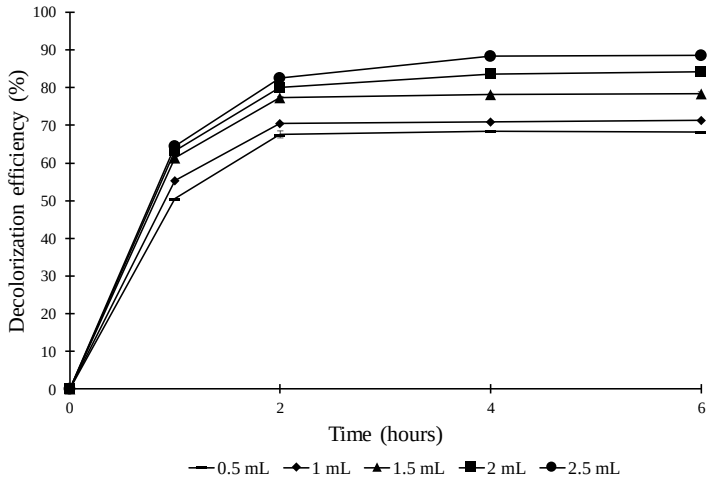

Figure 4. Effect of different enzyme concentrations on decolorization.

Enzyme activity was an important factor in decolorization. The decolorization efficiency mainly increased with enzyme concentrations [18]. Hsu *et al*., 2012 found that laccase from *Lentinus* sp. decolorized RBBR only 29% with 1 U/ml; however, by increasing laccase activity to 20 U/ml, the decolorization efficiency increased to 88% [14]. Moreover, laccase from *Lentinus* sp. showed better decolorization efficiency than that of a commercial *T. versicolor*. It was observed that high decolorization efficiency could be achieved if higher amount of laccase was used [22]; nevertheless, adding excess amount of crude enzyme for effluent decolorization in practical application might pose another environmental problem. This is because high protein contents or organic matters in the crude enzyme might require further wastewater treatment. Therefore, an appropriate amount of enzyme concentration should be applied.

3.5. Effect of mediators and metal ions on decolorization

As decolorization efficiency of 100 mg/l RBBR was relatively low, this initial concentration was chosen to test the effect of the mediators $CuSO_4$ and HBT, on improving decolorization. In addition,

chromium, which has usually been found in wastewater of textile industry, was selected to test the effect of metal ion on decolorization [1]. The result showed that addition of HBT (1, 5, 10 mM), $CuSO_4$ and $CrCl_4$ (10, 20, 30 mg/l) could increase decolorization efficiency, and $CuSO_4$ was better than HBT and $CrCl_4$ in enhancing decolorization, which could be because laccase is a copper-containing oxidase enzyme requiring Cu^{2+} to perform its active function. The maximum decolorization efficiency of the sample with $CuSO_4$ was 74% when using 30 mg/l $CuSO_4$, which was more than twice than that of the sample without mediator (Figure 5). Addition of HBT increased decolorization and 1 mM HBT was the best concentration to enhance decolorization up to 50%. However, decolorization efficiency did not correspond with increasing concentrations of HBT to 5 and 10 mM, which might be attributed to the inhibitory effect of excessive concentrations of mediators. Also, chromium could enhance decolorization up to 65% using 10 mg/l $CrCl_4$ and using higher concentrations of Cr^{3+} tended to decrease decolorization efficiency (Figure 6). Decolorization of the samples with 20 and 30 mg/l Cr^{3+} was 57% and 54%, respectively, at 6 hours of incubation. This indicated that decolorization efficiency was potentially affected by high concentration of Cr^{3+}, this could be because of the interaction of the high metal ion concentration interfered with the electron transport system of laccase contributing to its instability [26, 27]. Absorbance spectra of the samples with and without the mediators showed the peak at maximum wavelength around 592 nm (Figure 7). Degradation of the sample with $CuSO_4$ was the most effective in decolorizing RBBR; showing the lowest peak compared to the absorbance spectrum of the sample before decolorization.

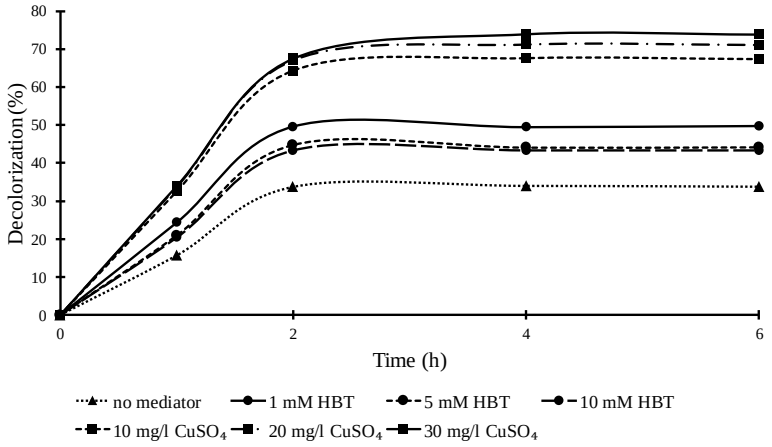

Figure 5. Effect of mediators, $CuSO_4$ and HBT on decolorization.

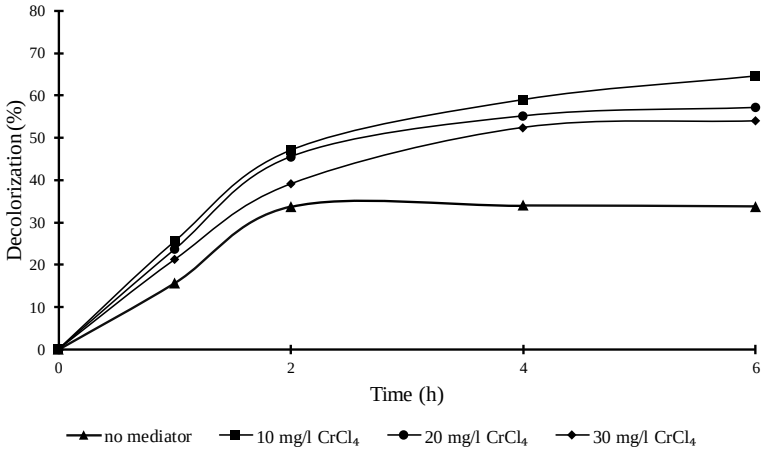

Figure 6. Effect of heavy metal, Cr^{3+} on decolorization.

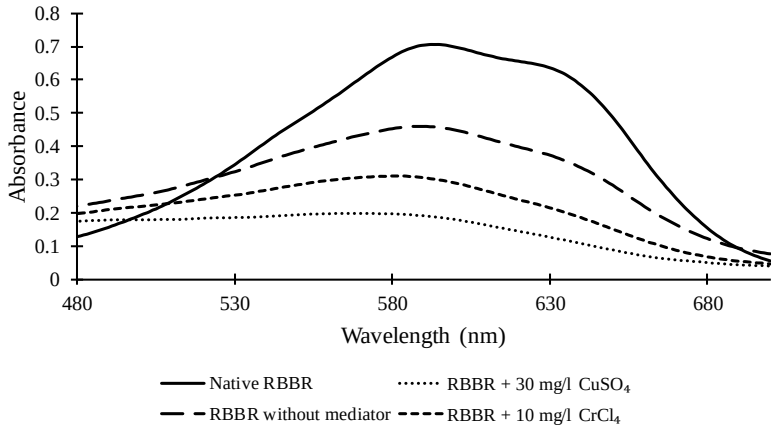

Figure 7. Absorbance spectra of the native RBBR and the decolorized RBBR in the presence and absence of mediators at 24 hours of incubation.

In the presence of mediators, laccase enzyme oxidizes low molecular weight mediators to stable radicals or redox mediators, then the radicals oxidize non-laccase substrates. Therefore, the mediator that has high affinity to the substrate, will enhance a laccase-mediator system increasing decolorization efficiency [26]. It has been reported that using redox mediators to enhance RBBR decolorization by adding Cu^{2+} to the culture medium, a maximum RBBR decolorization of 79% was obtained, compared to other metal ion mediators [26]. In addition, crude laccase (30 U/ml) produced from *P. ostreatus* strain 32 was able to decolorize 70% of remazol brilliant blue SN4R and the decolorization efficiency increased up to 90% in the presence of 0.16% ABTS mediator. However, inhibitory effect in decolorization appeared when an excessive amount of ABTS was applied [8]. Apart from using fungi for decolorization, cyanobacteria were also able to perform dye decolorization. Afreen *et al*., 2017 found that laccase from cyanobacteria, *Spirulina platensis* CFTRI, could decolorize 47% of 100 mg/l RBBR within 48 hours and decolorization efficiency increased almost twice in the presence of redox mediator syringaldehyde [3]. On the other hand, laccase produced from *L. polychrous* in the present study rapidly decolorized the dye up to 67% in the presence of $CuSO_4$ in a much shorter time which was within 2 hours, indicating high performance of the enzyme produced from the fungi. Among ligninolytic enzymes produced from the fungus, *Funalia trogii*, only laccase was responsible for RBBR decolorization, and decolorization was achieved without addition of hydrogen peroxide or redox mediator [5]. Similarly, RBBR decolorization in this study occurs without addition of H_2O_2 or $MnSO_4$, the main cofactor and substrate for lignin peroxidase and manganese peroxidase, indicating that laccase is the enzyme that plays a vital role in RBBR decolorization.

4. Conclusion

Ligninolytic enzyme produced by *L. polychrous* is mainly laccase, and it is responsible for RBBR decolorization. The crude laccase is effective in decolorization of RBBR at pH 3.0, while small decolorization is obtained at pH 6.0 and 7.0. At this optimum pH, around 78% of 20 mg/l RBBR is decolorized within 2 hours, which is relatively fast compared to some of the other fungal species or cyanobacteria, and decolorization decreases with increasing dye concentrations from 20 to 100 mg/l. By increasing concentrations of crude enzymes, the decolorization efficiency increases. In addition, decolorization efficiency increases more than twice in the presence of the mediator $CuSO_4$. However, increasing concentration of chromium tends to inhibit dye decolorization, thus contamination of chromium in the effluent of textile industry might decrease decolorization efficiency of the crude laccase. Using crude extract produced by *L. polychrous* to decolorize synthetic dyes is a simple, environmentally friendly and ecofriendly method which has high potential in the degradation of

several synthetic dyes and could also be applied for remediation of other aromatic and xenobiotic pollutants.

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