Remazol Brilliant Blue R Dye Decolourization by Laccase Produced by Pleurotus Sajor-caju via Solid-State Fermentation

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Abstract. The application of synthetic dyes in various industries improved the aesthetic properties of the products, but the discharge of dye-containing effluent into water bodies leads to serious environmental problems. Till date, colour removal from effluent is still a challenge due to the absence of economically attractive treatments. In this study, decolourization of a synthetic dye – Remazol brilliant Blue R (RBBR) using partially purified laccase from Pleurotus sajor-caju was investigated and compared to commercial laccase from Trametes versicolor. The findings show that crude laccase activity at 0.006 U/L produced by P. sajor-caju via solid-state fermentation did not decolourize RBBR (30 mg/L) well due to very low enzyme activity. However, the partially purified enzyme (0.07 U/L) is capable to decolourize RBBR solution up to 36% in 24 hours with an improvement of RBBR decolourization efficiency approximately by 12-fold. In comparison, when 0.1 U/L commercial laccase was used, only about 25% RBBR decolourization was achieved after 24 hours of incubation. This signifies that the laccase produced via solid-state fermentation has high enzyme activity, and can be used in bioremediation.

Keywords: Pleurotus sajor-caju; Synthetic dye; RBBR; Solid-state fermentation; Laccase.

1 Introduction

Synthetic dyes like azo, anthraquinone, heterocyclic, triphenylmethane or phthalocyanine dyes are widely applied in textile, paper making and cosmetic industries. Many of these dyes have found its way to discharging effluent, and the presence of these dyes in receiving water bodies leads to aesthetic pollution where it causes changes in water colour. Besides that, some dyes potentially form toxic aromatic amines that are mutagenic and carcinogenic in nature [1, 2]. These dyes are unusually resistant to degradation, and thus resulting in severe environmental problem [2].

To avoid excessive dyes from being discharged into receiving water bodies, physio-chemical decolourization treatment is usually employed as pre-discharge treatment. Several mechanisms involve in physio-chemical decolourisation are adsorption and precipitation approaches, chemical degradation or photodegradation. However, physio-chemical approach is laborious, time demanding and mostly not effective [2]. In view of these, biological decolourisation treatment using microbe or enzyme appears to be a more attractive approach to biodegrade and decolourize dyes in coloured-effluent [1, 2].

Enzymes that can degrade xenobiotic compounds are normally capable of degrading synthetic dyes in effluent. One class of the enzymes is the ligninolytic enzymes such as laccase. The enzyme can be produced from fungal or bacterial sources, and has low substrate specificity which can degrade a wide range of aromatic compounds. Laccase is versatile and has diverse applications where it could be applied in areas such as biological
pulping, bio-bleaching, waste detoxification and decontamination, decolourization of dyes and polyaromatic hydrocarbon, etc. [3].

In this study, laccase was produced in the laboratory from a white-rot fungus – *Pleurotus sajor-caju* (*P. sajor-caju*) via solid-state fermentation using rice husk as substrate. The efficiency of the produced enzyme in decolourization treatment of synthetic coloured-effluent containing Remazol brilliant blue-R (RBBR) was investigated, and its efficiency was compared to a commercial laccase.

2 Methodology

2.1 Chemicals

The Remazol Brilliant Blue R (RBBR, product code R8001) and commercial laccase from *Trametes versicolor* (product code 51639) was purchased from Sigma-Aldrich, USA. The commercial laccase was diluted into dilutions of desired enzyme activities before being used in decolourization experiments.

2.2 Production of Laccase via Solid-State Fermentation

The laccase was produced by *Pleurotus sajor-caju* via solid-state fermentation as previously reported by Ang [4]. Appropriate amount of moistened rice husk and 0.5% (w/w) fungal inoculum were loaded into a laboratory scale fermenter. The rice husk was moistened with solution supplemented with 0.5 mM copper sulphate, 10 g/L glucose and 0.01 g/L Tween 80 that are found to enhance laccase production in solid-state fermentation. Meanwhile, the fungal inoculum was prepared with the procedure as described by Ang [5]. After incubation for 84 hours, the enzyme was extracted from the solid substrate using 100 mM citrate buffer by agitating the content on a rotating agitator for 1 hour. The liquid portion containing crude enzyme was separated from residual rice husk particle and fungal biomass by centrifugation at 3500 rpm for 20 minutes (Eppendorf Centrifuge 5918 R, Germany). Following the production of enzyme, the crude enzyme filtrate was partially purified using Amicon Ultra-15 centrifugal filter (Millipore, USA). The activity of laccase was determined via laccase assay using 2, 2’-azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) as reagent. The concentrated solution was stored at -20°C prior to the decolourization assay.

2.3 RBBR Decolourization

The decolourization experiment was conducted using 100 ml reaction mixture composed of 30 mg/l of RBBR in 100 mM citrate buffer (pH 5) and various loading of laccase produced (0.06 – 0.1 U/L). The reaction mixture was incubated in a dark chamber at room temperature for 24 hours. The absorbance of RBBR remaining in the mixture was measured at an interval of 15 minutes with UV-VIS spectrophotometer at 595 nm, which corresponds to the maximum absorption wavelength of the dye. The percentage of decolourization of RBBR in reaction mixture was calculated using Equation (1).

\[
\text{Decolourization,} \% = \frac{A_i - A_f}{A_i} \times 100\%
\]

where \(A_i\) and \(A_f\) are the initial and final absorbance of the mixture, respectively.
2.4. Laccase Assay (Using ABTS)

The laccase activity was determined by means of UV-VIS spectrophotometry method using 30 mM 2, 2’-azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) reagent [6, 7]. The reagent was prepared from ABTS chromophore, diammonium salt (Calbiochem, product code 194430). ABTS reagent was diluted to a final concentration of 2.5 mM in reaction mixture using 100 mM of citrate buffer solution.

The changes in absorbance from the oxidation of ABTS at 420 nm were recorded with Secomam Prim Advances UV-VIS spectrophotometer at room temperature. Laccase activity was calculated from Beer-Lambert equation as shown by Equation (2). The molar extinction coefficient ($\varepsilon_{420}$) of ABTS ($36,000 \text{ M}^{-1}\text{cm}^{-1}$) was adapted from literatures [6-8]. The enzyme activity is defined as the amount of enzyme that oxidizes one μmol of ABTS per minute per litre, which activity is expressed in U/L.

$$A = \varepsilon \cdot c \cdot l$$

where $\varepsilon$ is molar extinction coefficient, $c$ is concentration of oxidized ABTS product (mol/L), and $l$ is the path length of light in cm.

| Table 1 Reaction mixture content of (a) Filtrate and (b) Blank for laccase assay |
|---------------------------------|---|
| **(a) Filtrate**                    |       |
| Reagent                          | Volume (μl) |
| ABTS (Final concentration = 2.5 mM) | 150       |
| Citrate buffer (100 mM, pH 4.8)   | 600       |
| Distilled water                  | 450       |
| Enzyme sample                    | 600       |
| **Total volume**                 | 1800      |

| **(b) Blank**                      |       |
| Reagent                          | Volume (μl) |
| ABTS (Final concentration = 2.5 mM) | 150       |
| Citrate buffer (100 mM, pH 4.8)   | 1200      |
| Distilled water                  | 450       |
| **Total volume**                 | 1800      |

3 Results and Discussion

The fungal laccase produced can decolourize RBBR solution. Laccase activity of crude enzyme filtrate possessed very low activity. After the crude enzyme filtrate was partially purified, its enzyme activity had been improved by 12-fold.
The percentage of laccase decolourization increases proportionally to incubation time at each activity (Figure 1). After 24 hours, the percentage of decolourization is ranged between 13 and 36%. In general, the initial decolourization efficiency (first 60 minutes) of RBBR increased with the increase in enzyme loading. The laccase loading at 0.1 U/L exhibited the highest RBBR decolourization followed by loading at 0.07 and 0.06 U/L. This indicates that the main determinant of RBBR decolourization efficiency is laccase loading.

![Figure 1](image)

**Figure 1** Percentage of RBBR decolourization by laccase produced via solid-state fermentation.

For comparison purpose, the experiment was repeated using commercial laccase from *Trametes versicolor*. The efficiency of initial RBBR decolourization of this commercial laccase increased as the enzyme loading increased from 0.1 to 1 U/L laccase loading (Figure 2). When the enzyme loading had increased to 2 U/L, RBBR decolourization efficiency declined significantly. The cause of this occurrence was not clear. It is interesting to mention that the decolourization efficiency of laccase produced via solid-state fermentation (35%) was better than the commercial laccase (25%) at 0.1 U/L after 24 hours. This observation is in congruence with the reported findings that fungal enzymes produced via solid-state fermentation have higher enzyme efficiency compared to the ones produced via liquid/submerged fermentation [9]. This might have attributed to the growing condition of solid-state fermentation that resembles the fungal natural habitat.
Conclusions
As a conclusion, laccase produced via solid-state fermentation decolourizes RBBR. Under the non-optimized condition, the produced enzyme was found to possess a decolourization efficiency of 36% when enzyme loading between 0.07 and 0.1 U/L was used. The laccase produced that was partially purified performed better with 35% dye decolourization as compared to the commercial laccase that had only 25% of decolourization ability after 24 hours. This signifies that the partially purified laccase produced via solid-state fermentation possesses high activity, and could be potentially used in various manufacturing industries or in bioremediation.

References


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