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# Biodegradation of remazol black B by extracellular fungal laccase from *Aspergillus oryzae* AM1101

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## Abstract

Extensive use of reactive dyes in textile industries and their discharge in water bodies as industrial waste without any treatment causes major ecological damage. This study was concerned with the development of environment friendly method for degradation of toxic dye to allow reuse of textile waste water for agricultural and industrial purposes. In this study, biological degradation of Remazol Black B by crude laccase from batch culture of *Aspergillus oryzae* AM1101was investigated. Rice bran was used as a medium for laccase production. Decolorization potential of laccase was investigated at different initial dye concentration, temperature, enzyme concentration and pH. Results showed that by using 200µl of enzyme maximum decolorization (76%) of Remazol Black B was achieved at initial dye concentration of 50mg/L, temperature 50˚C and pH 4.0. It was concluded that development of cost effective and eco friendly processes for textile dye decolorization could be achieved by optimization of experimental conditions.

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**Introduction**

Discharge of waste water from textile industries into water bodies is a major risk to ecosystem**.** This wastewater affects flora and fauna of aquatic ecosystem due to high biological and chemical oxygen demand. Lack of oxygen promotes development of dead zones (Sivaranjani *et al.*, 2013). Biomediated techniques have been gaining focus towards development of low cost and economical processes for treatment of wastewater before its discharge to aquatic system. Among these biomediated approaches, enzymatic degradation of reactive dyes is an economical and ecofriendly alternative for wastewater treatment as compared to other physicochemical processes due to no sludge formation and their rapid decolorization ability (Zeng *et al.*, 2011; Saratale *et al.*, 2011). Fungal laccases are considered more feasible for biodegradation of dyes due to their high redox potential and low substrate specificity (Kunamneni *et al.*, 2008).

This enzyme involved in various industrial processes i.e. in pulp bleaching (Moldes *et al.*, 2010), decolorization of textile dyes (Daassi *et al.*, 2012), pollutant detoxification (Khambhaty *et al.*, 2015) etc. For enzyme production, solid state and submerged fermentation have been used (Shraddha *et al.*, 2011). But solid state fermentation (SSF) is a better choice for enzyme synthesis as compared to submerged fermentation (SmF) because solid substrate in the form of agro industrial waste itself acts as a good source of carbon and little or no moisture content is required (Pandey *et al.*, 2000; El-Bakry *et al.*, 2015). In SSF, there is better oxygen circulation, no mechanical energy consumption, more concentrated metabolites are produced, purification procedures are not expensive, product recovery is easier, less wastewater is formed, less bacterial contamination and is a simple process (Eliasashvili *et al.*, 2008).

The present study was designed for efficient dye decolorization by crude laccase enzyme from locally isolated *Aspergillus oryzae* AM1101. The influence of parameters such as initial dye concentration, temperature, enzyme concentration and pH on the dye decolorization was evaluated in this study.

*Materials and methods*

*Dye*

Remazol Black B was obtained from local textile industry near Lahore. The stock solution (200mg/L) of this dye was prepared in double distilled water.

*Organism*

*Aspergillus oryzae* AM1101 was a choice of organism for laccase production. The fungal culture was subcultured on Potato dextrose agar slants after 2–3 weeks and maintained at 4°C.

*Laccase enzyme production by Aspergillus oryzae AM1101*

Solid substrate (Rice bran) was used for laccase production. This lignocellulosic material was moistened (50%) with Arulmani media (Arulmani *et al.*, 2007) and autoclaved it at 121°C for 15minutes. Cultural medium was inoculated with 5mm mycelial disc of 5-day-old fungal culture and incubated at 30°C for 5 days. After 5 days, enzyme was extracted by adding 100ml of 0.1M sodium acetate buffer and centrifuged at 6000 rpm for 15 minutes. After centrifugation the supernatant obtained was used as enzyme source.

*Laccase enzyme assay*

Enzyme assay was carried out by detecting the oxidation of ABTS in the reaction mixture spectrophotometrically at 420nm. The reaction mixture contained 0.005M ABTS, 0.1M sodium acetate buffer and suitable amount of crude enzyme (Birhanli and Yesilada, 2010).

*Enzyme unit*

Enzyme unit may be defined as the enzyme amount that oxidized 1µmol of substrate per minute at 30˚C (Alberts *et al.*, 2009).

*Enzymatic dye decolorization*

In 50mg/L of dye solution, particular amount of enzyme was added and incubated for 15, 30, 45 and 60 minutes. The pH and temperature was 4.0 and 30˚C respectively.

Rate of decolorization (%) was determined by monitoring the change in absorbance specrophotometrically at 596nm.

*Effect of initial dye concentration*

The dye decolorization activity of crude laccase was determined against different dye concentrations (50–200 mg/L). The experiment was performed at 30°C and pH 4.0. In this experiment, 100 µL of crude laccase was used.

*Effect of temperature on dye decolorization*

The effect of different temperature 10, 30, 50 and 70°C on dye decolorization activity was investigated at pH 4.0. The dye concentration and enzyme amount used were 50 mg/L and 100 µL, respectively.

*Effect of the enzyme amount on dye decolorization*

Different amounts of crude enzyme (50–200 µL) having 270 U/mL/min laccase activity, were used to determine the effect of enzyme amount on dye decolorization. The pH, temperature and dye amount was 4.0, 30°C and 50 mg/L, respectively.

*Effect of pH on enzymatic dye decolorization*

The effect of pH on decolorization activity was determined at pH 2.0, 4.0, 7.0,10 and 12 at 30°C. The pH of the reaction mixture was adjusted by using citrate phosphate buffer and Tris-HCl. Concentration of dye and amount of enzyme used were 50 mg/L and 100 µL, respectively.

*Data analysis*

All analytical results were sum of three replicates. All experimental data was showed in graphical format by using Origion Pro 8.0 software.

**Results and discussion**

*The effect of initial Remazol Black B concentration on decolourization*

Effect of Remazol Black B concentration on decolorization was observed by increasing the dye concentration from 50-200mg/L (Fig. 1).

It was observed that increase in concentration of dye from 50mg/L to 200mg/L resulted in the decreased in decolorization rate.

The enzyme decolourized about 49% of dye after 45 minutes when initial dye concentration was 50 mg/L. Further increase in dye concentration upto 200mg/L resulted in the decrease in decolorization rate to 26%. Our results are in close agreement with others workers that decolorization rate decreased with increase in dye concentration (Yesilada *et al.*, 2014; Ratanapongleka and Phetson, 2014).

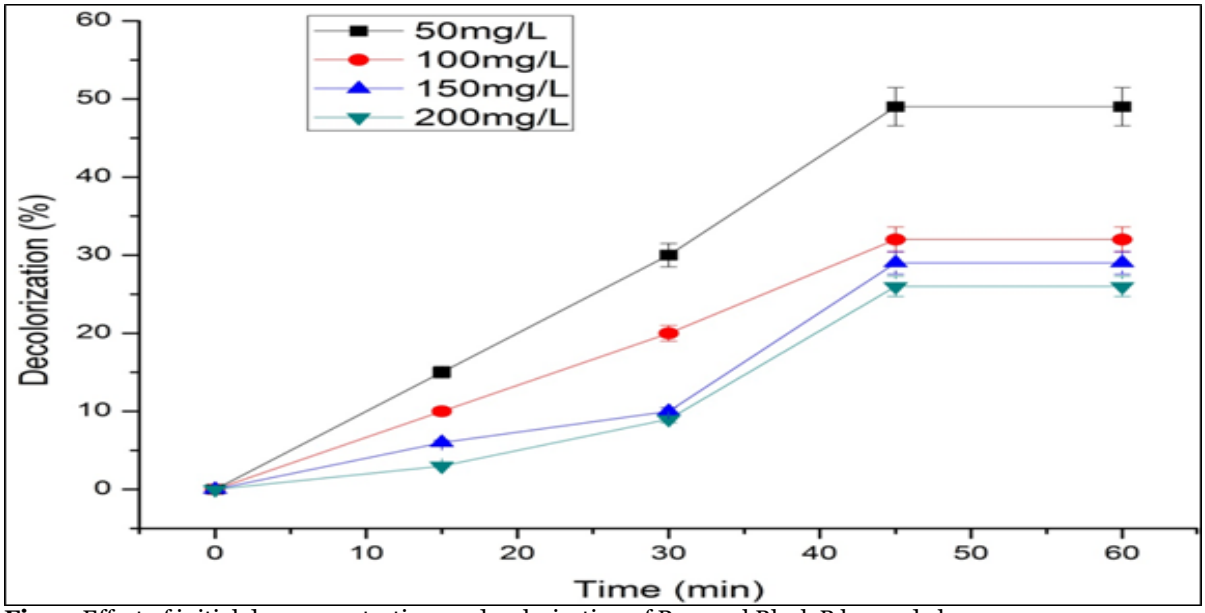


**Fig. 1.** Effect of initial dye concentration on decolorization of Remazol Black B by crude laccase.

*Effect of temperature on Remazol Black B decolorization*

Temperature has significant impact on dye decolorization. Effect of temperature on dye decolorization was investigated at 10, 30, 50°C and 70°C. It was resulted that maximum dye decolorization (55%) by crude laccase was achieved at 50°C after 45 minutes at pH 4.0 (Fig. 2). Further increase in temperature to 70°C resulted in the decrease in decolorization rate to 52%. These results are in close agreement with the previous work on decolorization of acid orange 51 by laccase produced by *Trametes trogii* (Daassi *et al*., 2013).

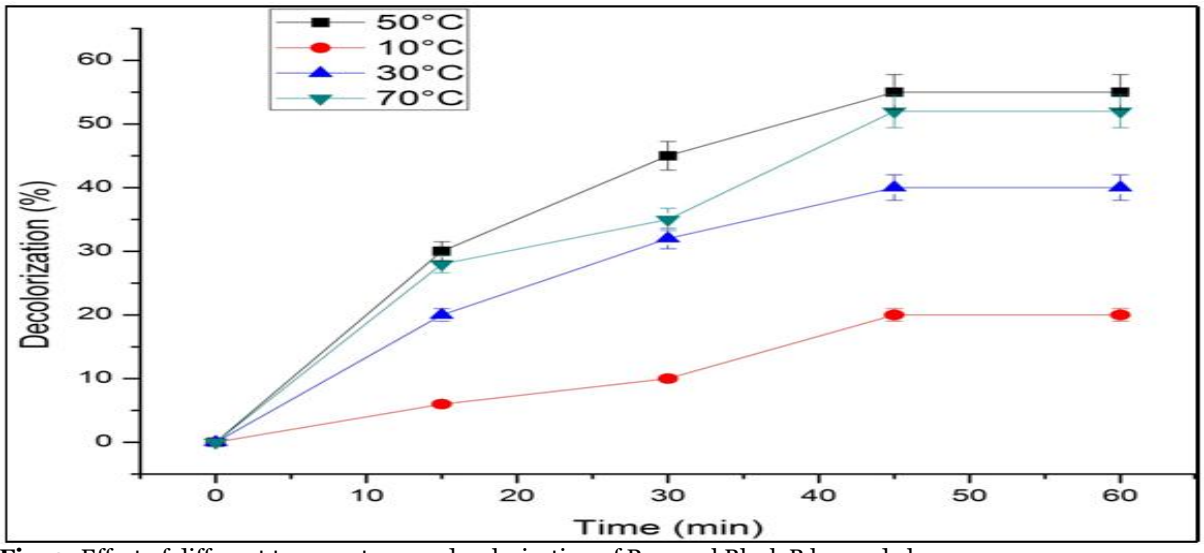


**Fig. 2.** Effect of different temperature on decolorization of Remazol Black B by crude laccase.

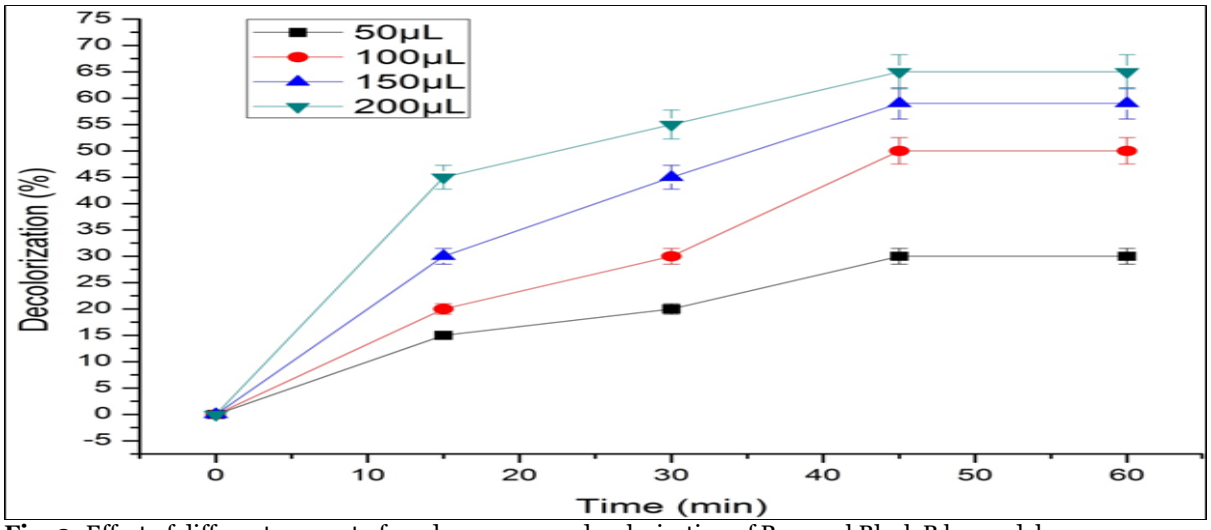


**Fig. 3.** Effect of different amount of crude enzyme on decolorization of Remazol Black B by crudelaccase.

*The effect of enzyme amount on Remazol Black B decolorization*

Amount of enzyme has significant effect on dye decolorization. The effect of crude laccase enzyme on decolorization rate was determined by increasing the enzyme amount from 50 µL to 200 µL. It was resulted that enzyme amount positively affect the dye decolorization and decolorization rate increased with increase in enzyme amount. The maximum decolorization (65%) was achieved after 45 minutes when 200 µL of enzyme was used (Fig. 3). Decolorization rate of dye at 50, 100 and 150 µL of crude enzyme was 30, 50, 59% respectively. Our findings that dye decolorization increased with increase in enzyme amount are in accordance with Yesilada *et al*., 2014.

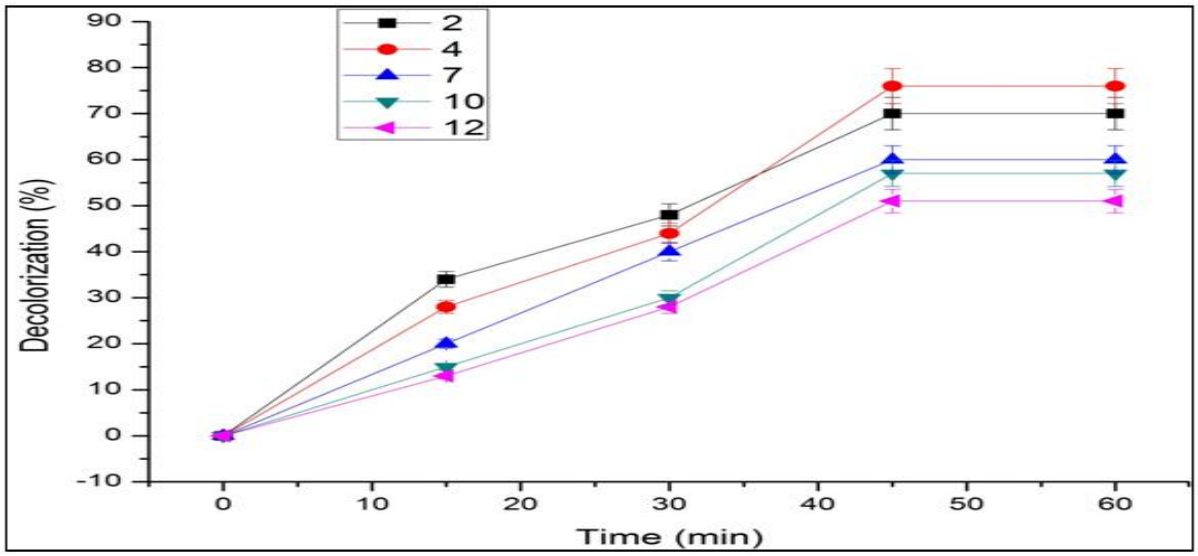


**Fig. 4.** Effect of different pH on decolorization of Remazol Black B by crude laccase.

The effect of pH on Remazol Black B decolorization

pH has a major and vital effect on dye decolorization efficiency (Ratanapongleka and Phetson, 2014). The effect of pH on decolorization of Remazol Black B was investigated at different pH 2, 4, 7, 10 and 12 at 30˚C after 15, 30 and 45 minutes (Fig. 4). Results showed that maximum decolorization (76%) was achieved at pH 4.0 after 45 minutes. Then decolorization activity of laccase was started to decrease and only 51% decolorization was observed at pH 12. These results are in accordance with other workers (Sarnithima *et al.*, 2009; Suwannawong *et al.*, 2010). According to these workers optimum pH for decolorization of Remazol brilliant blue (RBBR) and congo red were 3.0 and 4.0, respectively.

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**Conclusion**

This study concluded that crude laccase from *Aspergillus oryzae* AM1101 have excellent potential to decolorize Remazol Black B in short time period of 45 minutes. Results showed that 76% decolorization of Remazol Black B was achieved by optimization of experimental conditions.

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