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Enhanced Biodecolorization of Reactive Red 152 using *Polyporus rubidus* (MTCC 140): A Batch Culture Study

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A B S T R A C T

The discharge of dye-containing effluents from textile industries poses serious environmental and ecological concerns due to their toxicity, persistence, and aesthetic impact on receiving water bodies. In the present study, the biodecolorization potential of the white-rot fungus *Polyporus rubidus* (MTCC 140) was evaluated using Reactive Red 152 as a model textile dye under batch culture conditions. The effects of key operational parameters, including mycelial age, carbon source concentration, temperature, pH, and initial dye concentration, were systematically optimized to enhance decolorization efficiency. Maximum decolorization of 86% was achieved at 35 °C, pH 6.0, glucose concentration of 2 mg L^{-1}, initial dye concentration of 100 mg L^{-1}, and mycelial age of 6 days. Decolorization efficiency decreased at higher dye concentrations, indicating substrate inhibition. The results demonstrate the strong potential of *P. rubidus* for the biological treatment of dye-laden wastewater and highlight its applicability for eco-friendly remediation strategies.

Introduction

The textile industry is one of the largest industrial sectors worldwide and a major contributor to water pollution due to the extensive use of synthetic dyes during wet processing operations. A significant fraction of these dyes remains unfixed on textile fibers and is discharged into wastewater streams, imparting intense color, high chemical oxygen demand, and potential toxicity to aquatic ecosystems. Reactive dyes, in particular, are widely used because of their bright shades and excellent color fastness; however, their complex aromatic structures render them resistant to conventional physicochemical treatment processes.

Traditional treatment methods such as adsorption, coagulation–flocculation, and chemical oxidation often generate large volumes of secondary sludge and may lead to the formation of toxic by-products. Consequently, environmentally sustainable and cost-effective biological treatment strategies have attracted increasing attention. Microbial degradation, especially using fungi, offers significant advantages due to the production of extracellular oxidative enzymes capable of degrading a broad range of recalcitrant organic pollutants.

White-rot fungi are well recognized for their ability to

mineralize lignin and structurally related xenobiotic compounds, including textile dyes. Their non-specific enzymatic systems, such as laccases, manganese peroxidase, and lignin peroxidase, enable effective decolorization and detoxification of dye molecules. Several studies have reported successful dye removal using fungal species; however, optimization of operational parameters remains essential for achieving high treatment efficiency and scalability.

In this study, the biodecolorization capability of the white-rot fungus *Polyporus rubidus* (MTCC 140) was investigated using Reactive Red 152 as a model dye. The effects of critical parameters, including mycelial age, glucose concentration, temperature, pH, and initial dye concentration, were systematically evaluated to determine optimal conditions for maximum decolorization efficiency. The findings aim to contribute toward the development of sustainable biological treatment approaches for textile wastewater management.

Materials and Methods

Microorganism and Culture Conditions

The white-rot fungus *Polyporus rubidus* (MTCC 140) was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The culture was maintained on Sabouraud's Dextrose Agar (SDA) slants at 35°C. After seven days of incubation, a conidial suspension was prepared using sterile distilled water and used as the inoculum.

For inoculum development, 5 mL of the conidial suspension was transferred into 250 mL Erlenmeyer flasks containing 100 mL of Sabouraud's Dextrose Broth (SDB) and incubated at 35°C for six days on a rotary shaker at 130 rpm. The resulting fungal biomass was homogenized and used for batch decolorization experiments.

Dye and Experimental Setup

Reactive Red 152 was used as the model textile dye. Batch experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of fresh SDB supplemented with the desired dye concentration. Approximately 2 mL of homogenized fungal biomass was inoculated into each flask. The media were sterilized at 121°C for 20 min prior to inoculation.
The flasks were incubated on a rotary shaker at 130 rpm. Samples were withdrawn at regular time intervals and centrifuged at 1200 rpm for 20 min to remove biomass. The absorbance of the clear supernatant was measured at the maximum absorption wavelength (λmax = 472 nm) using a UV–visible spectrophotometer.

Calculation of Decolorization Efficiency

The percentage of decolorization was calculated using the following equation:

$$\text{Decolorization (\%)} = \frac{A_b - A_a}{A_b} \times 100 \quad \text{---------- (1)}$$

Optimization of Process Parameters

The influence of various operational parameters on dye decolorization was evaluated as follows:

Mycelial age: Cultures aged 2–6 days were tested.
Carbon source concentration: Glucose concentration was varied from 0.5 to 5 mg L^{-1}.
Temperature: Incubation temperature was varied from 20 to 60°C.
pH: Initial pH was adjusted between 3.5 and 7.0.
Initial dye concentration: Dye concentration was varied from 50 to 300 mg L^{-1} under optimized conditions.

All experiments were conducted in triplicate, and mean values were reported.

Table.1 Batch experiment conditions for decolorization of Reactive Red 152

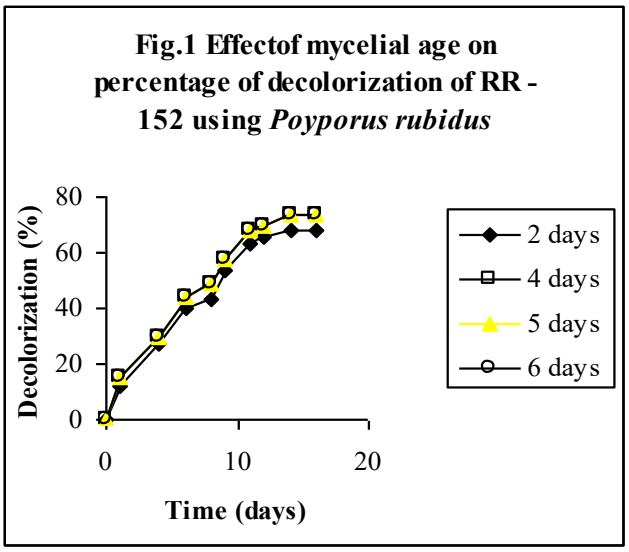
Parameter	Tested Range	Optimal Value
Mycelial age (days)	2, 3, 4, 5, 6	6
Temperature (°C)	20–60	35
pH	3.5–7	6
Glucose (mg/L)	0.5–5	2
Initial dye (mg/L)	50–300	100

Results and Discussion

Effect of Mycelial Age

The effect of mycelial age on the decolorization efficiency of Reactive Red 152 was evaluated using

fungal cultures aged between 2 and 6 days. As shown in Figure 1, a gradual increase in decolorization efficiency was observed with increasing culture age. Five-day-old cultures required approximately 48 h to achieve maximum decolorization, whereas six-day-old cultures achieved comparable decolorization within 24 h. Further increase in culture age did not result in a significant improvement in dye removal. Therefore, six-day-old mycelia were selected as the optimal inoculum age for subsequent experiments. Similar trends have been reported for fungal decolorization of synthetic dyes.

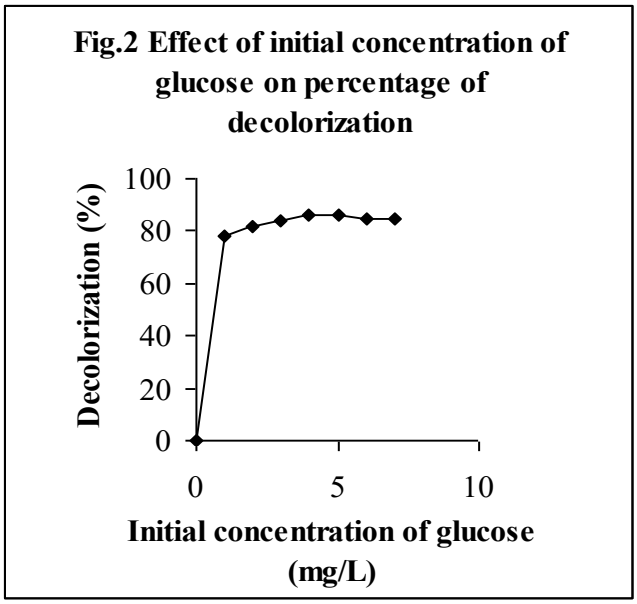


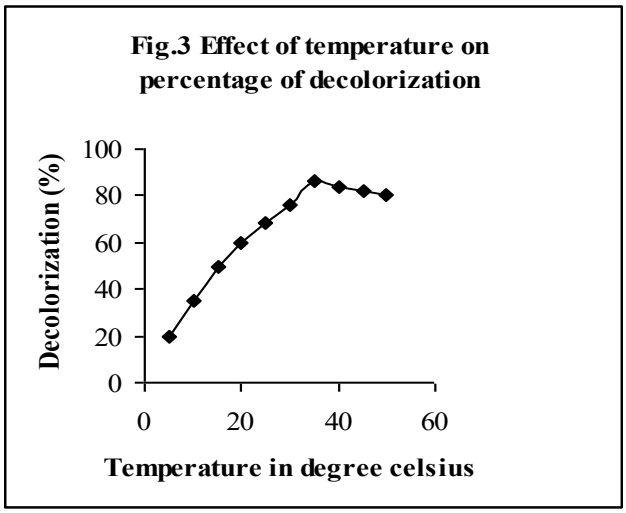
Effect of Carbon Source Concentration

The influence of glucose concentration on dye decolorization is presented in Figure 2. Glucose concentration was varied from 0.5 to 5 mg L^{-1}. Decolorization efficiency increased with glucose concentration up to 2 mg L^{-1}, beyond which no significant improvement was observed. The presence of an appropriate carbon source enhances fungal metabolic activity and enzyme production, thereby promoting dye degradation. Excess glucose may suppress oxidative enzyme synthesis, resulting in limited additional improvement.

Effect of Temperature

Temperature plays a critical role in fungal growth and enzymatic activity. The effect of temperature on dye decolorization was evaluated in the range of 20–60 °C (Figure 3). Maximum decolorization was observed at 35 °C. At lower temperatures, fungal growth was slower, leading to reduced decolorization rates. At temperatures above 35 °C, enzyme activity and cellular stability decreased, resulting in diminished dye removal. These findings indicate that *P. rubidus* exhibits optimal biodecolorization performance at mesophilic temperatures.



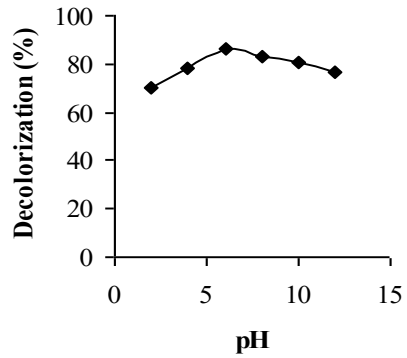


Effect of pH

The effect of initial pH on decolorization efficiency was investigated over a pH range of 3.5–7.0 (Figure 4). The fungus exhibited effective decolorization between pH 4.5 and 7.0, with maximum decolorization occurring at pH 6.0. At lower pH values, fungal growth was inhibited, whereas at higher pH values, fragmentation of

mycelial pellets was observed, leading to reduced decolorization efficiency. These results are consistent with the optimal pH range reported for white-rot fungal enzyme activity.

Fig.4 Effect of pH on percentage of decolorization



Effect of Initial Dye Concentration

The effect of initial dye concentration on decolorization efficiency was evaluated by varying the concentration from 50 to 300 mg L^{-1} under optimized conditions (35°C, pH 6.0, glucose 2 mg L^{-1}). As shown in Figure 5, decolorization efficiency increased with dye concentration up to 100 mg L^{-1}, achieving a maximum of 86% removal. Further increases in dye concentration resulted in a decline in decolorization efficiency, likely due to substrate inhibition and potential toxicity effects on fungal metabolism. High dye concentrations may also limit light penetration and enzyme accessibility.

Fig.5 Effect of initial concentration of dye on percentage of decolorization

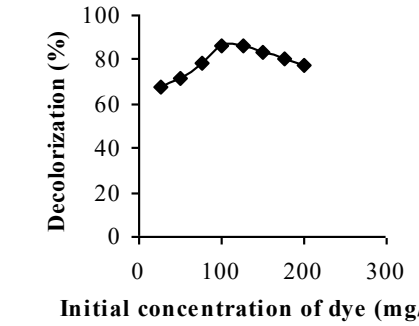


Table.2 Maximum decolorization achieved under optimal conditions

Parameter	Optimal Value	% Decolorization
Mycelial age	6 days	86
Temperature	35 °C	86
pH	6	86
Glucose	2 mg/L	86
Dye concentration	100 mg/L	86

The present study demonstrates the effective biodecolorization of Reactive Red 152 by the white-rot fungus *Polyporus rubidus* (MTCC 140) under batch culture conditions. Optimal decolorization (86%) was achieved at 35 °C, pH 6.0, glucose concentration of 2 mg L^{-1}, initial dye concentration of 100 mg L^{-1}, and mycelial age of 6 days. The observed decrease in decolorization efficiency at higher dye concentrations indicates substrate inhibition. These findings highlight the potential applicability of *P. rubidus* as a sustainable and eco-friendly biological agent for the treatment of dye-contaminated textile wastewater. Further studies focusing on continuous systems and enzyme characterization are recommended to enhance industrial applicability.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

N. Sangeetha: Investigation, analysis, writing original draft, S. Natarajan: Methodology, investigation,T. G. Nagulan: Conceptualization, methodology, writing

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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