Fungal laccase mediated Remazol Brilliant Blue R (RBBR) dye decolorization and degradation

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Abstract

The use of dyes is inevitable in various industries, but sustainable decolorization poses significant challenges that exhibit the need for microbial mediation. The present study investigated the potential of C. unicolor laccase for degrading Remazol Brilliant Blue R (RBBR) dye. The laccase treatment resulted in 91.8% decolorization of 100 ppm and 34.8% decolorization of 600 ppm RBBR after 12 hours. Optimization through one-variable-at-a-time (OVAT) analysis revealed optimal conditions: 20 U/ml laccase concentration, pH 5 and a 10-hour treatment time. To examine the interactive effects of temperature and dye concentration on degradation, response surface methodology (RSM) with central composite design (CCD) was applied.

The predicted maximum decolorization of 80% at 275 ppm RBBR concentration and 40°C closely aligned with the experimental result of 85%. UV-vis spectroscopy showed a reduction in absorbance at 591 nm, while LC-MS identified degradation products including hydroxyphenyl-containing compounds (m/z 216 and m/z 222) and sulfanilic acid derivatives. The current study contributes to the advancement of efficient, sustainable strategies for RBBR dye bioremediation that might address critical environmental concerns related to dye pollution and will further enhance the development of bioremediation technologies for industrial applications.

Keywords: Central Composite Design, *Cerrena unicolor*, Laccase, RBBR decolorization, Response Surface Method.

Introduction

Remazol Brilliant Blue R (RBBR) dye stands as a prominent member of the diazo dye family, widely employed across various industrial sectors such as textiles, paper and leather processing due to its vibrant hue and ease of application[7]. The extensive use of RBBR brings forth significant environmental concerns, primarily owing to its persistent nature and potential toxicity[16]. During RBBR synthesis and dyeing, the colored effluent generated is discharged into aquatic or ecosystems where RBBR has the propensity to accumulate within fish tissues, disrupting the delicate balance of aquatic habitats[19]. Moreover, the resilience of RBBR to conventional treatment methodologies underscores the imperative to explore alternative remediation approaches[20].

RBBR is an anthracene derivative and represents an important class of toxic and recalcitrant organ pollutants. There are several reports on decolouration of RBBR by laccases. Conventional treatments for RBBR degradation , encompassing physical and chemical methods like adsorption and photo degradation, have been reported[6]. Yet, these methodologies often proved to be ineffective, economically unfeasible and may inadvertently contribute to secondary pollution[17]. Conversely, biodegradation emerges as a promising solution for RBBR mitigation, offering the potential for efficient and environmentally benign dye removal[20].

In search of efficient biodegradation strategies, research interest has converged on laccase-producing organisms i.e. white rot fungi, particularly *C. unicolor*. These fungi exhibit the capability to secrete ligninolytic enzymes including laccases. Laccases, renowned for their broad substrate specificity and environmental compatibility, present a promising avenue for RBBR decolorization and detoxification [9]. Building upon previous research endeavors, this research aimed use of laccase from *C. unicolor* (OR713083.1) for decolorization of RBBR. One variable at a time (OVAT) approach was used for optimization of RBBR decolorization by considering enzyme concentration, pH and treatment time.

Statistically designed experimental models were applied to optimize the decolorization parameters. Response surface methodology is an experimental approach to identify the optimum conditions for a multivariable system. In this study, RSM has been applied for the optimization of parameters, temperature and dye concentration. Biotransformation of RBBR was performed and was confirmed exploiting analytic techniques including UV/VIS spectroscopy and HPLC- MS. UV–Vis spectroscopy analysis was used to examine the changes in dye structure and to reveal the enzymatic degradation process. The dye degradation intermediates were revealed by HPLC- MS analysis.

This study attempts to determine use of purified laccase enzyme of *C. unicolor* in decolorization and degradation of Remazol Brilliant Blue R (RBBR). Also, the process parameters of dye decolorization were studied with OVAT approach and response surface method. The degradation

products were analyzed by UV/VIS spectroscopy and HPLC- MS techniques.

Material and Methods

Chemicals and microorganism: RBBR was purchased from Sigma-Aldrich. The white rot fungus *C. unicolor* strain (OR713083.1) was isolated, identified and used for laccase production.

Laccase Enzyme preparation: The purified laccase enzyme was obtained using *C. unicolor* fermentation followed by acetone precipitation[2], gel exclusion chromatography and SDS PAGE method[5]. The 20 U/ml of the purified laccase was used in all the assays performed.

Dye Decolorization Studies: RBBR dye decolorization using *C. unicolor* was studied by preparing stock solution of 100 ppm in sodium acetate buffer at pH 5. The reaction mixture containing 5ml of 100 ppm dye added with 1ml of pure laccase (10U/ml) was incubated for 12 h under static conditions at 30^0 C. % decolorization was monitored spectrophotometrically by measuring the reduction in absorbance of the dye solution at 595 nm wavelength [13] and calculated according to the following formula:

$$D\ (\%) = (A_0-A_1)/A_0 x100$$

where D is the decolorization efficiency (%), A_0 is RBBR absorption before pure laccase treatment and A_1 is the residual RBBR absorption after pure laccase treatment.

Samples were drawn after each one hour, put in spectrophotometric cuvettes in which reaction was stopped by adding sodium azide to the medium.

Optimization of RBBR decolorization by One Variable At A Time (OVAT) approach: Optimization of RBBR dye decolorization was preliminary conducted by one variable at a time OVAT approach using five factors including enzyme concentration (5- 30 U/mL), pH (3-9), temperature (200C-70 ^{0}C), treatment time (0-12 hours) and dye concentration (100 – 600 ppm). Initially the effect of dye concentration on decolorization was studied by considering different dye concentrations (100 – 600 ppm). The other parameters were studied using the stock solution of 300 ppm. To study the effect of enzyme concentration on dye decolorization, reaction mixtures containing 5 ml RBBR (300 ppm) treated with 1ml of different concentrations of pure laccase ranging from 5-30 U/mL were incubated for 12 h at 30^{o}C.

To study the effect of pH, temperature and treatment time on decolorization, 5 mL dye solution (300 ppm reaction volume) added with 1 ml of 20 U enzyme concentration was incubated for 12 hours at 30^{o}C. All experiments of the dye decolorization were performed in triplicate.

Optimization of RBBR decolorization using Response Surface Methodology: Response surface methodology (RSM) was employed to optimize decolorization conditions for RBBR. Central composite design (CCD) was chosen for the optimization of RBBR decolorization process by pure laccase. The optimization parameters included temperature (10-70°C) and dye concentration (50-500 ppm) [11]. The % decolorization was calculated and data was analyzed by ANOVA statistics. The significance of model, percent prediction of dye decolorization and lack of fit were tested. Mean, standard error of mean and ANOVA with Post hoc Tukey's HSD were done using SPSS ver. 24. The optimization of experiments was done using Design expert ver. 7.

Identification of degradation products using UV -Visible spectroscopy and HPLC- MS: UV–Vis spectroscopy analysis was used to examine the changes in dye structure to reveal the enzymatic degradation process [1]. To examine the enzymatic degradation of the RBBR, the adsorption of the dye samples before and after laccase treatment (12 h) was scanned to obtain comparative UV–Vis spectra. In order to know whether RBBR dye was decolorized or degraded, the dye decolourized solution was subjected to comparative HPLC- MS method to identify the dye degradation intermediates.

Remazol brilliant blue R standard stock solution (Control) was prepared by dissolving 10 mg Remazol brilliant blue R into a 10 mL clean and dried volumetric flask, add about 7 mL of acetate buffer pH 5.00 and sonicate to dissolve it completely. Make volume up to the mark with acetate buffer pH 5.00 (1000 PPM). Inject on HPLC and chromatogram was recorded. Treated dye was mixed with 2ml of methanol prior to HPLC analysis. The elution of sample was done isocratically using a C18 reversed phase column (RPC - Kromasil C18) and analyzed by using mobile phase consisting of 70% of acetonitrile and 30% water (HPLC grade). The flow rate of mobile phase was 1.0 ml/min and the UV detector was set at 591 nm.

Results and Discussion

Dye Decolorization Studies: The RBBR dye was checked for possible decolorization by using purified laccase from *C. unicolor*. Maximum decolorization of RBBR by laccase was observed (greater than 90%) after 12 h treatment. There is evidence that RBBR cannot be decolourised without redox mediators just by pure fungal laccases[15]. However, in the present investigation, RBBR dye was decolorized by pure laccase in absence of mediator, indicating this laccase as a potential catalyst for further RBBR decolourisation.

Optimization of RBBR decolorization by OVAT approach: Selected variables (different enzyme dose, pH, temperature, treatment time and dye concentration) were studied for decolorization of RBBR dye by laccase using the OVAT approach. This study indicated that dye decolorization was strongly influenced by the studied variable discussed as follows:

Decolorization of RBBR dye by using purified laccase enzyme produced by *C. unicolor* was evaluated for 12 h incubation with different concentrations of RBBR dye ranging from 100-600 ppm. The result is shown in figure 1. The maximum decolorization reached 91.8% at 100 ppm after incubation for up to 12 h. Along with dye removal, the decolorization rate was also calculated to understand the effect of the initial dye concentration on decolorization. It has been reported that the decolorization rate may be increased with increasing dye concentration. In this study, although the percent decolorization decreased with increasing RBBR concentrations, the decolorization rate increased with increasing dye concentration. At a concentration of 200 ppm, the maximum decolorization rate of RBBR was obtained at 7.65 ppm/h, while it reached 20.93 ppm/h at 500 ppm after 12 h.

Maximum decolorization efficiency (> 80%) was observed at 100–300 ppm RBBR within 12 h. The decolorization percent of RBBR decreased significantly with increasing dye concentration, reaching 34.8 % at 600 ppm, which was probably due to the dye binding to the enzyme's active site or interfering with the enzyme's substrate binding. High dye concentrations can also lead to the formation of enzyme-dye complexes that are inactive.

Effect of pH on Remazol Brilliant Blue-R -Dye decolorization: The effect of pH on the percent decolorization of RBBR dye using a laccase enzyme concentration of 20 U/mL was investigated across a range of pH values (keeping treatment time of 12 hours). The results highlight the pH sensitivity of laccase mediated RBBR dye decolorization, with optimal decolorization occurring in the acidic pH range. At pH 4 and pH 5, the percent decolorization was notably high, with values of 76.5% ± 0.1% and 81.2% ± 0.3% respectively than pH 3, indicating effective dye degradation under acidic conditions. However, as the pH increased to neutral and alkaline ranges, a significant decrease in decolorization percentage was observed.

At pH 6, the percent decolorization dropped to 32.1% ± 0.2% and at pH 7, it further decreased to 15.1% ± 0.1% (Figure 2). Under alkaline conditions at pH 8, the decolorization efficiency was considerably diminished, with percent decolorization of 10.1± 0.1% indicating a significant reduction in dye degradation capability. The Tukey's HSD found significant difference in all the values. pH 5 gave significantly higher decolorization percentage. This result suggests that the most favorable pH for decolorizing the RBBR dye could be at the pH range of 4−5.

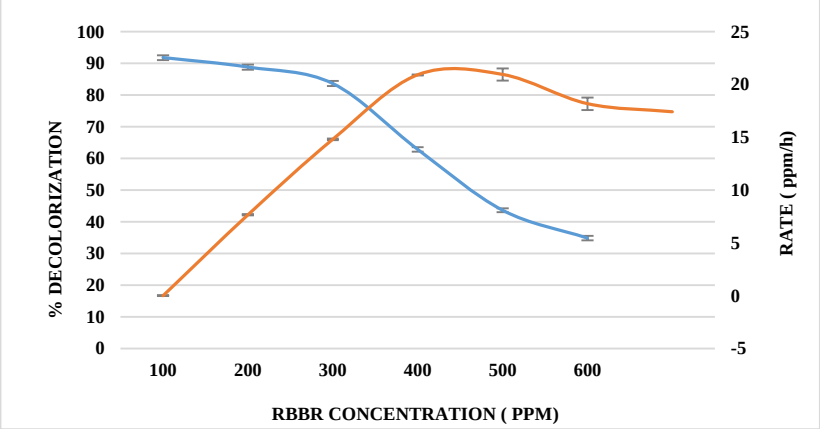


Figure 1: Effect of purified laccase (*C. unicolor) on* initial concentration of RBBR decolorization, inset with the corresponding percent decolorization and decolorization rate

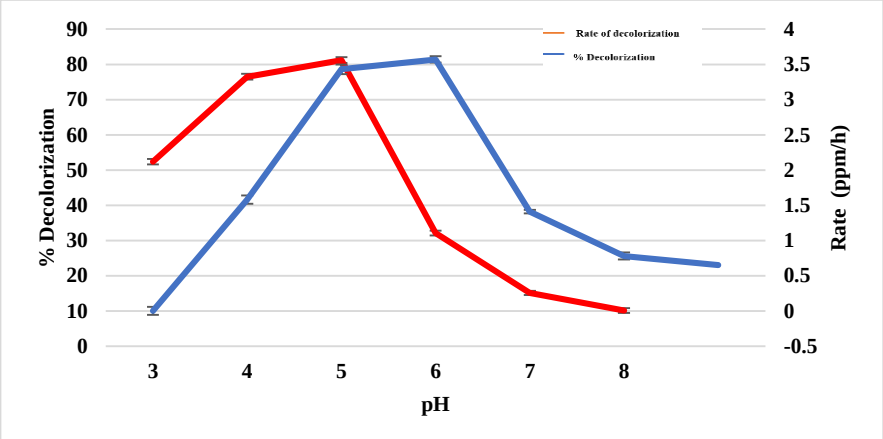


Figure 2: Effect of pH on decolorization of RBBR dye by purified laccase from *C. unicolor*

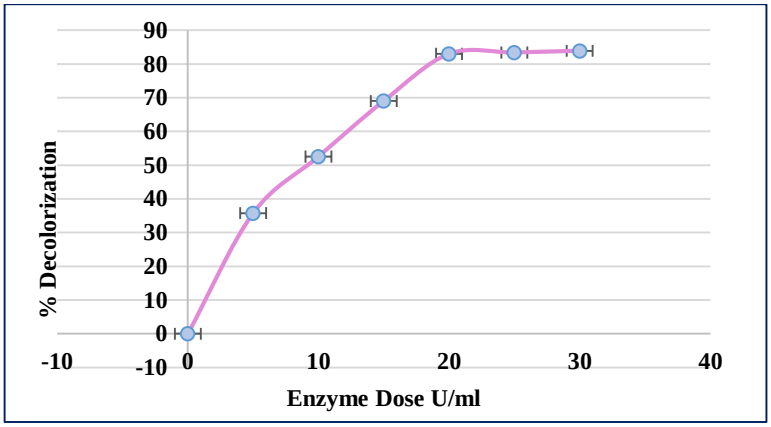


Figure 3: Effect of purified laccase enzyme (*C. unicolor)* concentration on % decolorization of RBBR

At pH 5, the maximum decolorization rate of RBBR was obtained as 3.43 ppm/h, while it reached 0.78 ppm/h at pH 8 after 12 h. Maximum decolorization efficiency (> 80%) was observed at pH 5 within 12 h. The decolorization percent of RBBR decreased significantly after increase in pH from 6 to 8, which may be probably due to the ionization of amino acids at the enzyme's active site, causing irreversible denaturation of the enzyme. This structural change renders the enzyme inactive, as it can no longer catalyze reactions effectively.

This result supports the previous observation that the highest decolorization efficiency of RBBR dye mediated by the white rot fungus KRUS-G strain is when the culture medium was conditioned at pH 4[18]. Laccase is generally stable across many pH gradients and pH 5 found to be optimal[12]. One factor at a time approach for optimal pH is a robust method and is used widely[8] to reduce the number of runs in design of experiments.

Effect of initial enzyme concentration on Remazol Brilliant Blue-R -Dye decolorization: The percent decolorization of RBBR dye using purified laccase enzyme was investigated at various enzyme doses ranging from 5 U/mL to 30 U/mL (keeping pH 5 and treatment time of 12 hours). As the enzyme dose increased, there was a notable increase in the % decolorization of the dye. At an enzyme dose of 5 U/mL, the percent decolorization was recorded at 35.75% ± 0.1%. Further increments in enzyme dose to 10 U/mL, 15 U/mL and 20 U/mL resulted in considerable enhancements in % decolorization. At 10 U/mL, % decolorization reached 52.53 % ± 0.3%, while at 15 U/mL and 20 U/mL, it increased even further to 69.1% ± 0.3% and 82.9% ± 0.2% respectively. % decolorization shows steady decolorization of 83.3 % at 25 and 83.8 at 30 U/ml (Figure 3).

ANOVA test followed by Tukey's HSD found that there was significant difference in all the percent dye reduction values, interpreting that 20 U/mL was the optimum concentration showing highest dye decolorization. Patel et al[14] found 542 ppm reduction of laccase at 1U/mL by laccase from *Coriolopsis caperata*, when incubated for 1 hour. Hong et al[4] found 6 strains of different white rots which could completely degrade RBBR in 3 days. The RBBR degradation has not been studied using laccase produced by *C. unicolor*.

These results suggest a clear dose-dependent relationship between laccase enzyme concentration and the decolorization efficiency of the RBBR dye. Higher enzyme doses corresponded to greater decolorization percentages, indicating the effectiveness of laccase in degrading the dye.

The Effect of Temperature on decolorization of RBBR: The enzyme activity of purified laccase from *C. unicolor* was strongly influenced by reaction temperature and its effect on dye decolorization. The temperature range chosen to explore RBBR decolorization by purified laccase was 20–70 °C. The effect of increase in temperature on RBBR decolorization is shown in figure 4. The decolorization percentage was low at 20 °C (18.5 %) and significantly increased at 30°C (45.53%). By increase in temperature from 30 to 40 °C, the change in decolorization percentage obtained was 79.99%. When the temperature exceeds 50 °C, the decolorization percentage declined significantly.

The % decolorization obtained at 70°C was only 13.1 %, indicating temperature over 50°C as not suitable for laccase enzyme activity to decolorize RBBR. The optimum reaction temperatures of laccases from other white-rot fungi can reach between 30–70 °C. In this study, RBBR decolorization percentage reached the maximum at 40 °C, beyond which the percentage decolorization decreased sharply.

Effect of treatment time on decolorization of RBBR: The decolorization of RBBR dye using laccase enzyme at a concentration of 20 U/mL was investigated over a period of 12 hours (keeping pH 5). The process exhibited a gradual increase in decolorization percentage over time, starting from 0% at the onset and reaching 85.2% by the end of the experiment. In the initial hour, 6.9 % decolorization was observed which significantly accelerated in subsequent hours, with decolorization percentages of 17%, 23.1% and 29.6% observed at hours 2, 3 and 4 respectively. As time progressed, the rate of decolorization continued to rise,

indicating the effectiveness of laccase in degrading the RBBR dye. Notably, a substantial leap in decolorization was noted at the 6th hour, with a percentage of 48.1% achieved, signifying an enhanced enzymatic activity.

This trend persisted, with the decolorization percentage exceeding 61.7 % by the 7th hour and surpassing 81% by the 9th hour. However, the rate of decolorization appeared to plateau in the latter stages of the experiment, with marginal increases observed towards the end (Figure 5).

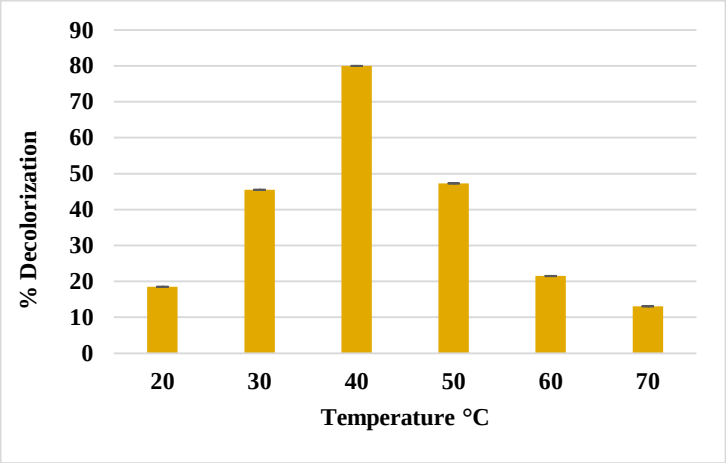


Figure 4: Effect of Temperature on decolorization of RBBR dye by purified laccase from *C. unicolor*

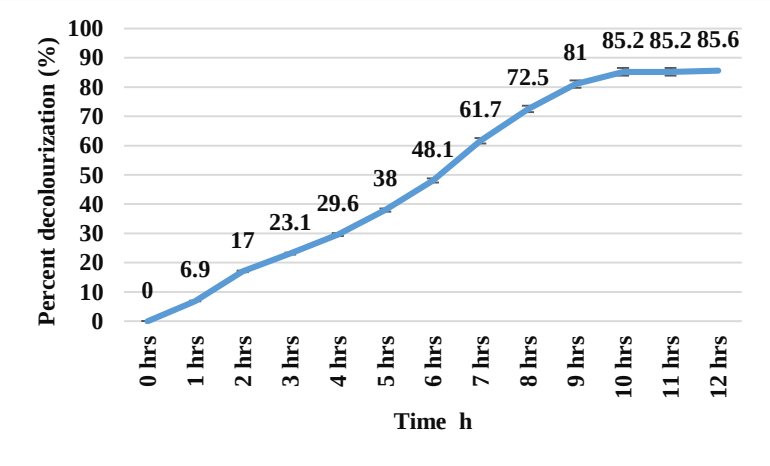


Figure 5: Effect of treatment time with purified laccase (*C. unicolor*) on % decolorization of RBBR.

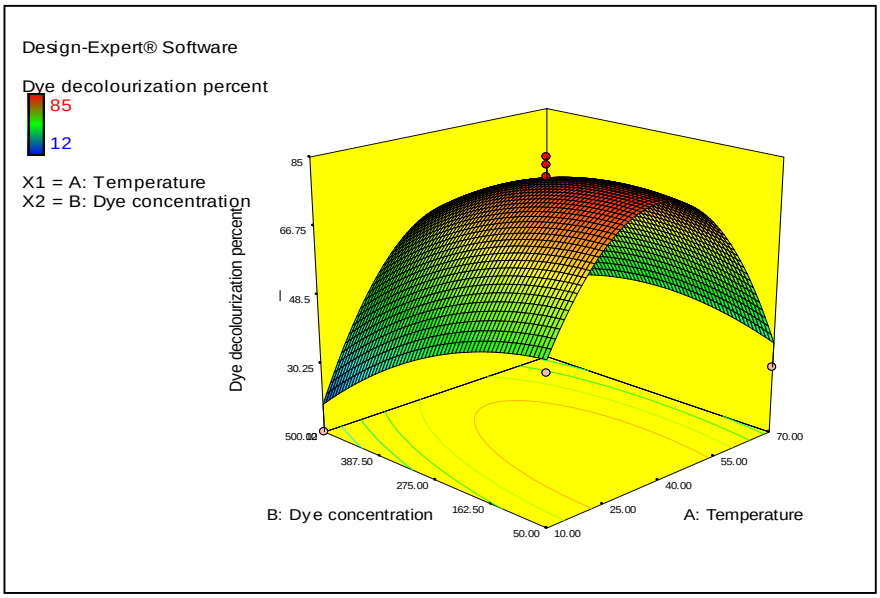


Figure 6: Optimization of dye decolorization using dye concentration and temperature as variable parameters.

At 12th hour, decolorization percentage of 85.2% suggested maximum degradation of the RBBR dye under the employed conditions. Tukeys HSD found that there was significant difference in the percent decolorization of RBBR dye in all the treatment times except for 10 to 12 hrs of treatment time. Hence, 10 hours of treatment was optimal for the decolorization at given concentration of dye and enzyme.

Naseem et al[10] found 64.1 % RBBR degradation after 5 hours of laccase treatment, which was comparatively more than the current activity. Chhabra et al[3] found 95 % azo dye reduction with immobilized laccase in 5 days. This activity was lower as compared to current findings where 85.2 % reduction of dye was found in 10 hours. The activity of the laccase is certainly influenced by the fungal origin and conformation, which need to be researched to find competitive laccase activity. It has been reported that the decolorization rate may be increased with increasing treatment time.

RSM optimization of variables using Central composite design: The RBBR dye decolorization optimization study, employing response surface methodology (RSM) with central composite design (CCD), included experimental parameters as temperature (°C) and dye concentration (ppm). Notably, at 40°C and 275 ppm, actual percent decolorization closely mirrored predicted values, reaching up to 85% against the predicted 80 % (Table 1). However, deviations appeared under varied conditions. For example, at 10°C and 500 ppm, actual decolorization dropped to 12%, notably below the predicted 20%.

Similarly, extremes in temperature (0°C and 82°C) and low or high dye concentrations yielded considerable differences between actual and predicted values. This suggests the model's limitations in extreme conditions. Nevertheless, the analysis shed light on critical factors influencing decolorization and provided a basis for optimization.

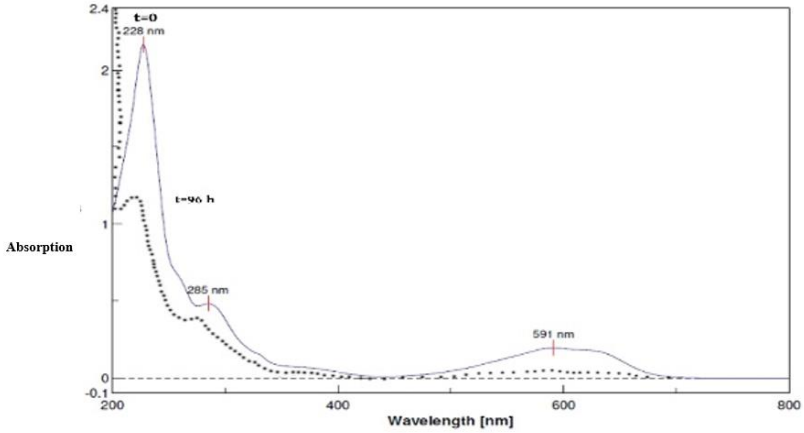
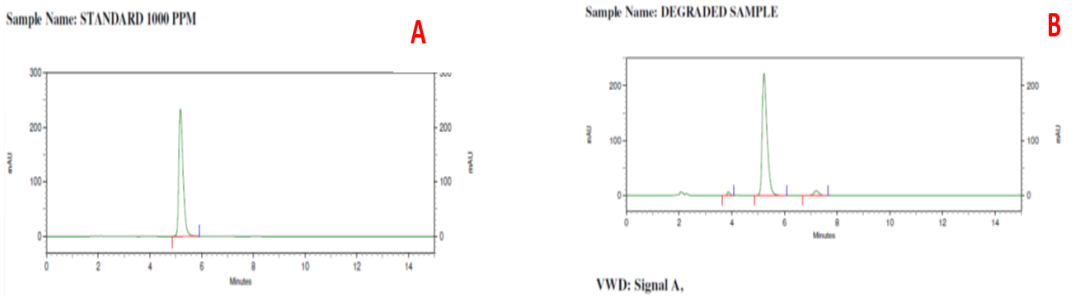


Figure 7: UV–Vis spectrum of Remazol Brilliant Blue R (RBBR) solution (800 mg/L, Control) before degradation (solid line). Degradation products of RBBR (dotted line) treated by purified laccase from *C. unicolor*.

Table 1
Optimization experiments using Central composite design to find the actual and predicted values of dye decolorization.

Temperature (°C)	Dye concentration (ppm)	% Decolorization (actual)	% Decolorization (Predicted)
40	275	85	80
40	275	83	80
40	593.198	65	54
10	50	50	53
40	275	70	80
10	500	12	20
0	275	20	15
82	275	18	8
70	500	25	36
40	275	78	80
40	275	80	80
70	50	30	37
40	0	85	80

Final Equation in Terms of Actual Factors:
RBBR degradation = 28.62159+2.90727 * Temperature - 0.014276 * Dye concentration +1.22222E-003 * Temperature * Dye concentration - 0.040550 * Temperature^2 - 1.31488E-004 * Dye concentration^2.



VWD: Signal A, 591 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Remazol brilliant blue R	5.18	45724168	1.31	5648
Totals		45724168		

VWD: Signal A, 591 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Degradation product 1	3.89	621524	1.02	2804
Remazol brilliant blue R	5.25	40856276	1.29	5352
Degradation product 2	7.20	1985623	0.99	5973
Totals		43463423		

Figure 8: HPLC profile of RBBR degraded by laccase from *C. unicolor* (A) RBBR untreated (B) RBBR treated with laccase

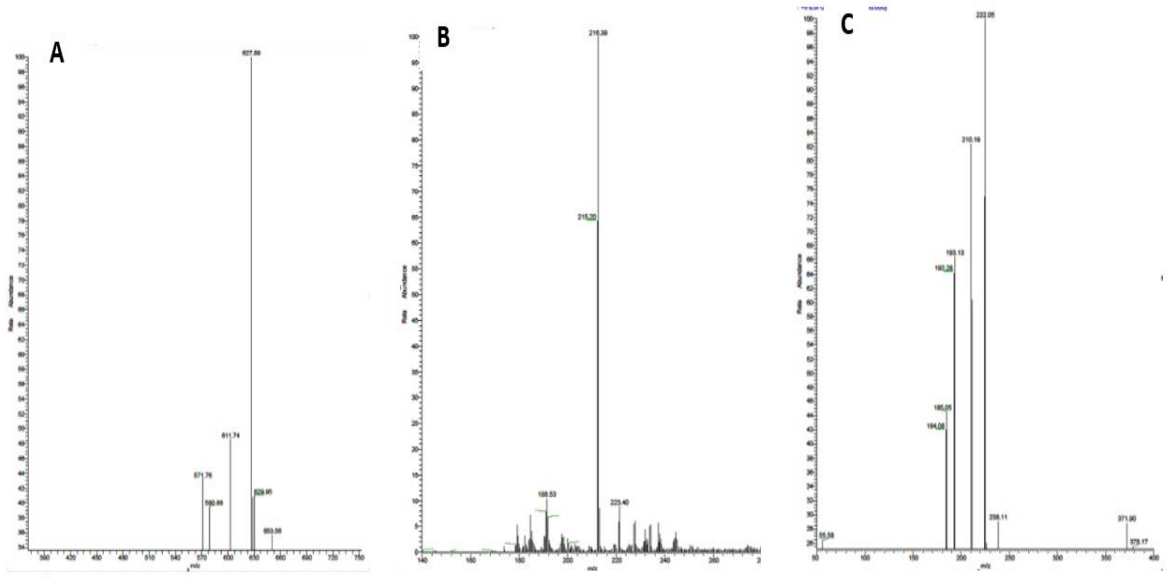


Figure 9: The mass spectra of RBBR and its degradation products (A) RBBR (control)- m/z 627 (retention time 5.18 min) (B) Intermediate 1- m/z 216 (retention time- 3.89 min) (C) Intermediate -2 m/z 222 (retention time 7.20 min)

Table 2
Optimization of dye decolorization by using response surface methodology-

Source	Sumof squares	df	Mean Square	F Value	p-value Prob > F	Significance
Model	9399.3	5	1879.86	20.51	0.0005	significant
A-Temperature	2.47E-03	1	2.47E-03	2.69E-05	0.996	
B-Dye concentration	529.43	1	529.43	5.78	0.0472	
AB	272.25	1	272.25	2.97	0.1285	
A^2	8452.28	1	8452.28	92.21	< 0.0001	
B^2	255.86	1	255.86	2.79	0.1387	
Residual	641.62	7	91.66			
Lack of Fit	506.82	3	168.94	5.01	0.0767	not significant
Pure Error	134.8	4	33.7			
Cor Total	10040.92	12				

Final Equation in Terms of Actual Factors:
RBBR degradation = 28.62159+2.90727 * Temperature - 0.014276 * Dye concentration +1.22222E-003 * Temperature * Dye concentration - 0.040550 * Temperature^2 - 1.31488E-004 * Dye concentration^2

Moving forward, refining the model to better accommodate extreme conditions may enhance accuracy, thus facilitating improved dye decolorization processes. The optimized dye decolorization response surface graph is shown in figure 5. The ANOVA (Table 2) shows the significance of the model and its individual factors, along with interaction effects and quadratic terms. The model's F value of 20.51 suggests that the model as a whole is significant, as the associated p-value is much lower than the typical significance level of 0.05.

This indicates that the chosen factors, their interactions and quadratic effects collectively contribute to the response variable i.e. the percent decolorization. It was observed that temperature (A) and dye concentration (B) cumulatively have significant effects. Temperature exhibits a negligible effect with an almost negligible F-value and a significantly high p-value, rendering it statistically insignificant. Conversely, dye concentration (B) demonstrates a significant effect on the decolorization process, as indicated by its relatively high F-value and a p-value of 0.0472, which is below the 0.05 threshold.

The interaction term AB representing the combined effect of temperature and dye concentration shows a moderately high F-value but a p-value of 0.1285, which exceeds the typical threshold. Therefore, this interaction might not significantly affect the decolorization process within the range of values tested.

Moreover, both quadratic terms, A^2 and B^2, exhibit substantial impacts on decolorization, as evident from their high F-values and very low p-values. This suggests that the relationship between temperature and dye concentration and the decolorization process is nonlinear within the experimental range. In the residual analysis, the lack of fit test assesses whether the model adequately represents the data compared to pure error. The lack of fit F-value of 5.01 yields a p-value of 0.0767, indicating that the lack of fit is not significant at the 0.05 significance level. This suggests that the model adequately fits the data, although there might be some minor inadequacies that are not accounted for.

3D response plot of effect and interaction between temperature and dye concentration for percentage decolorization was plotted (Figure 6). In this study, decolorization percentage of dye increases up to 40^0C. Further increases in temperature decreased decolorization of dye. On the other hand, the downward trend was observed when dye concentration increases from 250 ppm, the percentage of decolorization decreased.

UV- HPLC analyses: Decolorization of RBBR by purified laccase was accompanied by changes in its absorption spectrum (Figure 7). The visible peak (591 nm) disappeared at the end of the experiment (96 h) indicating maximum decolorization which implies the cleavage of the chromophore group after the enzymatic treatment, where significant color removal can be observed. HPLC analysis was performed in order to confirm the degradation of the RBBR. HPLC profile of the untreated dye showed single peaks at retention time 5.18 min while the products formed by treating RBBR with laccase showed peaks at retention times 3.39 and 7.20 min. Differences in the HPLC profiles of RBBR and the metabolites formed confirm the dye degradation. HPLC profile of laccase treated dye (degraded dye) and the control sample (RBBR dye) showed just one peak in common (5.18 min) which indicated that the other two peaks in the HPLC profile of the metabolites were a consequence of degradation of the dye. (Figure 8)

To detect the products of metabolism of RBBR, mass spectra analyses were carried out to investigate the intermediates and the final transformation products of RBBR after pure laccase treatment. Figure 9 showed major peak of RBBR (control) with a retention time of 5.18 min (m/z 627) shown as a major peak. The presence of different molecules at the retention times 3.89 and 7.20 suggested the formation of two intermediate products with m/z 216 and 222 respectively

Conclusion

The present study reported that *C. unicolor* laccase is a powerful tool for the decolorization of textile dye (RBBR). Optimization of decolorization was studied by means of one-factor-at-a-time method (OVAT) and central composite design (CCD). The classical one-factor-at-a-time method indicated that percentage and rate of decolorization were affected by enzyme dose, pH, treatment time and dye concentration. The CCD design was useful in obtaining maximum decolorization (85%) at 40^0C of 275 ppm.

Increase in temperature above 40^0C affected enzyme capacity to decolorize RBBR. Based on the intermediates identified by HPLC-MS, laccase catalysed effective RBBR decolorization and degradation and split the RBBR molecule into two intermediates. These encouraging results went a step forward towards the industrial application of C. *unicolor* laccase. This research contributes to the development of sustainable and efficient strategies for mitigating dye pollution in aqueous condition, paving the way for future advancements in bioremediation technologies by using laccase from *C. unicolor*.

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References

1. Ameenudeen S., Unnikrishnan S. and Ramalingam K., Statistical optimization for the efficacious degradation of reactive azo dyes using Acinetobacter baumannii JC359, *Journal of Environmental Management*, **279**, 111512 **(2021)**

2. Bryjak J. and Rekuć A., Effective purification of Cerrena unicolor laccase using microfiltration, ultrafiltration and acetone

precipitation, *Applied Biochemistry and Biotechnology*, **160**, 2219-2235 **(2010)**

3. Chhabra M., Mishra S. and Sreekrishnan T.R., Immobilized laccase mediated dye decolorization and transformation pathway of azo dye acid red 27, *Journal of Environmental Health Science and Engineering*, **13 (1)**, 38 **(2015)**

4. Hong C.Y., Kim HoYong K.H., Jang SooKyeong J.S. and Choi InGyu C.I., Screening of outstanding white rot fungi for biodegradation of organosolv lignin by decolorization of remazol brilliant blue R and ligninolytic enzymes systems, *Journal of the Korean Wood Science and Technology*, **41(1)**, 19-32 **(2013)**

5. Isanapong J., Suwannoi K., Lertlattanapong S. and Panchal S., Purification, characterization of laccase from Pleurotus ostreatus HK35 and optimization for congo red biodecolorization using Box–Behnken design, *3 Biotech*, **14(3)**, 73 **(2024)**

6. Joshni T.C. and Subramaniam K., Enzymatic degradation of azo dyes-a review, *International Journal of Environmental Sciences*, **1(6)**, 1250-1260 **(2011)**

7. Kataria J., Rawat H., Tomar H., Gaurav N. and Kumar A., Azo Dyes Degradation Approaches and Challenges: An Overview, *The Scientific Temper*, **13(02)**, 384-400 **(2022)**

8. Kumar R., Kaur J., Jain S. and Kumar A., Optimization of laccase production from Aspergillus flavus by design of experiment technique: Partial purification and characterization, *Journal of Genetic Engineering and Biotechnology*, **14(1)**, 125-131 **(2016)**

9. Kumar V.V., Kirupha S.D., Periyaraman P. and Sivanesan S., Screening and induction of laccase activity in fungal species and its application in dye decolorization, *African Journal of Microbiology Research*, **5(11)**, 1261-1267 **(2011)**

10. Naseem S., Rawal R.S., Pandey D. and Suman S.K., Immobilized laccase: an effective biocatalyst for industrial dye degradation from wastewater, *Environmental Science and Pollution Research*, **30(36)**, 84898-84917 **(2023)**

11. Njoku C.N. and Otisi S.K., Application of central composite design with design expert v13 in process optimization, In Response Surface Methodology-Research Advances and Applications, IntechOpen **(2023)**

12. Novoa C., Dhoke G.V., Mate D.M., Martínez R., Haarmann T., Schreiter M., Eidner J., Schwerdtfeger R., Lorenz P. and Davari M.D., KnowVolution of a fungal laccase toward alkaline pH, *ChemBioChem*, **20(11)**, 1458-1466 **(2019)**

13. Nursyam H., Kautsarah Y.I.M., Prihanto A.A., Hayati R.L. and Muyasharoh H., Biosynthesis and Characterization of Silver Nanoparticles-Synthesized using Extracts of Mangrove Sonneratia caseolaris leaves, *Res. J. Chem. Environ*., **28(2)**, 37-43 **(2024)**

14. Patel A.M., Patel V.M., Pandya J., Trivedi U.B. and Patel K.C., Evaluation of Catalytic Efficiency of Coriolopsis caperata DN Laccase to Decolorize and Detoxify RBBR Dye, *Water Conservation Science and Engineering*, **2(3)**, 85-98 **(2017)**

15. Peng Q., Wu Y., Adil B., Wang J., Gu Y., Yu X., Zhao K., Zhang X., Ma M. and Chen Q., Optimization of Laccase from Ganoderma lucidum Decolorizing Remazol Brilliant Blue R and Glac1 as Main Laccase-Contributing Gene, *Molecules*, **24(21)**, 3914 **(2019)**

16. Rajput R.B., Jamble S.N. and Kale R.B., A review on TiO2/SnO2 heterostructures as a photocatalyst for the degradation of dyes and organic pollutants, *Journal of Environmental Management*, **307**, 114533 **(2022)**

17. Rathi B.S. and Kumar P.S., Sustainable approach on the biodegradation of azo dyes: a short review, *Current Opinion in Green and Sustainable Chemistry*, **33**, 100578 **(2022)**

18. Syafiuddin A. and Fulazzaky M.A., Decolorization kinetics and mass transfer mechanisms of Remazol Brilliant Blue R dye mediated by different fungi, *Biotechnology Reports*, **29**, e00573 **(2021)**

19. Tkaczyk A., Mitrowska K. and Posyniak A., Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: A review, *Science of the Total Environment*, **717**, 137222 **(2020)**

20. Tripathi M., Singh S., Pathak S., Kasaudhan J., Mishra A., Bala S., Garg D., Singh R., Singh P. and Singh P.K., Recent strategies for the remediation of textile dyes from wastewater: a systematic review, *Toxics*, **11(11)**, 940 **(2023)**.

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