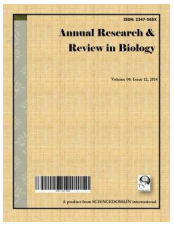

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Decolorization of Cibacron Blue 3G-A Dye by *Agaricus bisporus* CU13 Laccase - Mediator System: A Statistical Study for Optimization via Response Surface Methodology

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMO designed the study, wrote the protocol, performed the experimental and statistical analysis and wrote the first draft of the manuscript. Authors AME, MAE and MMH contributed in results evaluation and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: the present study aims to optimize Cibacron Blue 3G-A decolorization as a model dye through laccase enzymatic biocatalysis presenting the role of HBT as a redox mediator via RSM approach.
Study Design: RSM using Central Composite Design (CCD) was used in order to determine the most effective variables levels in Cibacron Blue 3G-A decolorization and to investigate their interactions.
Place and Duration of Study: Department of Microbial Chemistry, Genetic Engineering and Biotechnology Research Division, National Research Centre (NRC), Cairo, Egypt, between August 2017 and January 2018.
Methodology: The evaluation of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude

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laccase was conducted through different trials using a 1.5 mL reaction mixture containing different concentration of crude laccase, Cibacron Blue 3G-A, and HBT in 0.1 M sodium citrate buffer (pH 4.5) at room temperature for different incubation periods.

Results: Hydroxybenzotriazole (HBT) as a mediator enhanced Cibacron Blue 3G-A decolorization levels significantly, where decolorization percentage caused by laccase enzyme alone were 11.92 and 23.78%, whereas that caused by laccase HBT mediator system under the same conditions were 43.43 and 76.34% after 1 and 22 h of incubation, respectively. HBT concentration, dye concentration, enzyme activity, and incubation time were chosen as study variables to optimize Cibacron Blue 3G-A dye decolorization through RSM approach via central composite design (CCD). The optimum conditions for Cibacron Blue 3G-A decolorization were found to be under using 0.50 U/mL of *Agaricus bisporus* CU13 laccase, 92.19 ppm of Cibacron Blue 3G-A, and 1 mM of HBT in order to get decolorization percentage of 29.29% in 35 min.

Conclusion: *Agaricus bisporus* CU13 crude laccase was used as a biocatalyst to decolorize Cibacron Blue 3G-A in presence of HBT as a mediator through utilizing the response surface methodology approach. HBT concentration, dye concentration, enzyme activity, and incubation time affects the decolorization levels considerably.

Keywords: Bio-decolorization; optimization; laccase; anthraquinone dyes.

1. INTRODUCTION

Numerous types of dyes are expansively utilized in textile industries to color the textile products. The dyes firstly adsorbed to textile fibers and then interact with it. Depending to the degree of dye fixation, around 10–20% of the primary dye load is released as effluent causing an extremely colored wastewater that resulting in serious environmental problems [1,2].

Different studies about the using of traditional physical and chemical methods like oxidative process, filtration, adsorption, precipitation, etc. for dyes elimination are available [3]. Recently, numerous studies have revealed that fungal enzymes are capable to detoxify and decolorize different kinds of industrial dyes [4]. Nevertheless, dyes treatments using enzymatic approaches are not used regularly yet in textile industries. Enzymatic approaches for dye decolorization and degradation have many advantages such as low costs, low energy, have a little impact on the ecological systems and easy to control [5].

Laccase (EC 1.10.3.2) is a member of oxidative enzymes group that catalyze the oxidation of a wide range of substrates including phenolic compounds, aromatic amines, and polyphenols [6,7]. Preceding studies have revealed that the laccases substrate specificity range could be expanded by adding redox mediators that act as electron shuttles like 1-hydroxybenzotriazole (HBT) [8] 2,2-azinobis(3-ethylbenzothiazoline-6-Sulfonate) (ABTS) [9], violuric acid [10] and polioxometalates [11].

The application of classical methods for experimental optimization via altering only one factor for each trial and fitting the remaining factors has various shortcomings, as it point up the effect of every parameter independently through a enormous amount of experimental trials, on the other hand it doesn't reflect on the interaction effect between diverse parameters [12]. Alternatively, the application of statistical methods in biotechnology answers the troubles originated from one factor per time approach for selection of effectual factors and considering the applied parameters interaction in order to optimize and improve the process settings [13].

Response surface methodology (RSM) application in process of textile waste matter treatment is an approach to reduce process time, costs and variability, in addition to improve effluents decolorization process. Furthermore, the factors influencing the decolorization experimentation are optimized, identified, and the probable antagonistic or synergic exchanges between factors can be estimated [14]. RSM has been widely considered in different biotechnological applications but not many reports are offered in for optimization of dye degradation via enzymatic catalysis through RSM [15].

Cibacron Blue 3GA is an important dye utilized in enzymes purification through affinity chromatography techniques after its immobilization to a porous support like agarose [16]. Cibacron Blue 3GA is one of anthraquinone anionic dyes which operates as a P_2-

purinoceptor competitor [17] and hinders the discharge of stimulus-evoked glutamate from cortical tissue of rat brain [18]. It also restrains β-lactamases (OXA-1 and OXA-2) and utilized to monitor the ligands binding to OXA-1 β-lactamase by observing the fluorescence of tryptophan [19].

The current study aims to optimize Cibacron Blue 3G-A decolorization as a model dye through laccase enzymatic biocatalysis presenting the role of HBT as a redox mediator via RSM approach.

2. MATERIALS AND METHODS

2.1 Chemicals and Enzyme Source

Cibacron Blue 3G-A dye (Sigma B1064) was obtained from textile industries research division, National Research Centre, Egypt. 2, 2´-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) the laccase substrate was purchased from Sigma (Canada). 1-Hydroxybenzotriazole hydrate (HBT) was supplied by Aldrich (5482). All other chemicals and reagents used in this work were of high purity and analytical grade. Culture filtrate from *Agaricus bisporus* CU13 fungal strain was used as laccase source under our previously studied conditions for laccase production [20].

2.2 Laccase Activity Assay

Laccase activity was assessed spectrophotometrically by Agilent Carry-100 UV-Vis Spectrophotometer, Germany, using ABTS (0.3 mM) solution in 100 mM citrate buffer (pH 4.5) as substrate. The reaction conditions was similar to that described previously by Othman et al. [20]. One unit of laccase was defined by the amount of enzyme necessary to oxidize 1 µmol of the ATBS per min. Protein content was calculated by the method described by Bradford [21] using bovine serum albumin (BSA) as the standard protein. All experimentations were made in triplicates, and the mean value was presented ± standard deviation.

2.3 Cibacron Blue 3G-A Decolorization

The evaluation of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase was conducted through different trials using a 1.5 mL reaction mixture containing different concentration of crude laccase, Cibacron Blue 3G-A, and HBT in 0.1 M sodium citrate buffer (pH 4.5) at room temperature for different incubation periods.

The absorbance peak of the decolorization mixture was recorded at the definite desired incubation time for each trial taking into account the conversion of the dye molecules to other compounds absorbing at different wavelengths, and the decolorization percentage was calculated as: $Decolorization\ (\%) = Abs_0 - Abs_t / Abs_0 \times 100$, Where Abs_0 is the sum of initial absorbance peak and Abs_t was the sum of final absorbance peak at different time intervals. All experiments were preformed in triplicate, and control samples which include the identical reaction mixture without enzyme were also done.

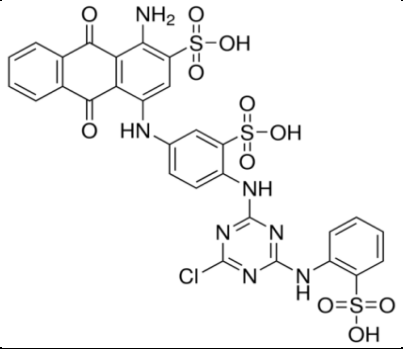
2.4 Experimental Design for Dye Decolorization via RSM

RSM using Central Composite Design (CCD) was used in order to determine the most effective variables levels in Cibacron Blue 3G-A decolorization and to investigate their interactions. In the current study, four effective variables were selected to optimize the decolorization process. These factors are laccase activity, Cibacron Blue 3G-A concentration, incubation time, and HBT concentration. Each variable was considered in different rational five coded levels (2, 1, 0, -1, -2). The total number of experimental runs was obtained as: $2^k + 2k + X_0$, where k is the number of variables (4) and X_0 is the experiments repetitions at the center point (6). So, a 30 experimental runs were performed according to CCD presented in Table 2. The second order polynomial equation mentioned below was used to estimate the correlation between variables and the resulted response.

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i + \Sigma\beta_{ij} X_i X_j$$

Where Y is the predicted response, X_i, X_i^2, X_j are coded values for used variables; β_0 is constant; β_i is the linear effect; β_{ii} is the squared effect and β_{ij} is the interaction effect. The second-order model is extensively used in response surface methodology due to its extremely flexibility, can employ a wide diversity of functional forms, and there is a significant practical experience representing that second-order models work well in solving actual response surface problems [5]. The obtained results were analyzed using the statistical and graphical analysis software (Design Expert Version 7.0.0) to analysis

Table 1. Molecular formula and chemical structure of Cibacron Blue 3G-A

Name	CAS No	Chemical structure	Molecular formula	Molecular weight
Cibacron Blue 3G-A	84166-13-2		$C_{29}H_{20}ClN_7O_{11}S_3$	774.16

regression of the data obtained and to estimate regression equation coefficient. Table 2 presents the coded and actual values for these codes and the central composite design and the actual and predicted experimental results.

3. RESULTS AND DISCUSSION

3.1 Effect of HBT-laccase Mediator System on Cibacron Blue 3G-A Decolorization

Laccase usually is able to oxidize a wide variety of substrates including phenols and aromatic amines through reducing the oxygen into water molecules via a multi-copper system. On the other hand, a number of substrates having a high redox potential are not accessible to direct oxidation by laccase. For that, laccase mediator systems can be the competent key to solve this obstacle, where mediators like HBT operate as a redox shuttle to minimize the redox gap between laccase potential and desired compound [8]. This because sometimes the dye has higher redox potential than that of laccase Cu 1 center or due to the dye steric hindrance [1].

In current study, we used HBT as a mediator in order to evaluate its effect on Cibacron Blue 3G-A decolorization process, where HBT is extensively considered as laccase redox mediator and has established to be an exceptionally efficient mediator in the occurrence of laccase [1]. Results obtained declared that the Cibacron Blue 3G-A decolorization percentage caused by laccase enzyme alone were 11.92 and 23.78%, whereas that caused by laccase-HBT mediator system under the same conditions were 43.43 and 76.34% after 1 and 22 h of incubation respectively (Fig. 1). From these results, it is clear that Cibacron Blue 3G-A decolorization was significantly influenced by HBT-laccase mediator system with a raise in percentage of decolorization by around 3.6 and 3.2 fold over the equivalent sample with laccase enzyme alone after 1 and 22 h of incubation, respectively (Fig. 1). In this connection, a further detailed experiment was conducted to emphasis the HBT-laccase mediator system on Cibacron Blue 3G-A decolorization (Fig. 2). Data presented in Fig. 2 showed that after only 10 min, about 7.09 and 13.48% of the dye were decolorized using laccase and HBT-laccase mediator system respectively, which referring a good laccase capability system toward Cibacron Blue 3G-A decolorization. The decolorization percentage has increased gradually as a function of reaction time to attain about 9.33 and 29.07% after 30 min (Fig. 2).

In agreement, Othman et al. [8] stated that HBT presence was compulsory for Reactive Black 5 decolorization with *Myceliophthora thermophila* covalently immobilized laccase on functionalized multiwalled carbon nanotubes membranes, because no decolorization was observed without using HBT as a mediator. They concluded that enzyme activity and mediator were the most important factors that affect decolorization process [8].

3.2 Explanation of Regression Analysis

From the results of the previous experiment it is clear that HBT as a redox mediator is very effective in Cibacron Blue 3G-A dye decolorization, for that reason optimization of HBT concentration is crucial for a good impacted decolorization process as agreed by other

previous studies [5]. In addition, dye concentrations, enzyme activity, and incubation time were chosen as study variables to optimize Cibacron Blue 3G-A dye decolorization through RSM approach as indicated in the central composite design. The actual and predicted values of Cibacron Blue 3G-A decolorization percentage were cited in Table 2.

The quadratic model was employed to interpret the statistical correlation among the independent parameters and the dependent reaction result. The correlation between Cibacron Blue 3G-A decolorization and the design variables was expressed in the following mathematical equation in terms of coded values.

Decolorization percentage = 23.835 + 1.015833 A - 0.5975 B + 2.989167 C + 1.319167 D + 1.33125 AB + 1.075 AC - 0.13625 AD - 0.17375 BC + 0.5 BD - 0.31375 CD -0.33583 A^2 - 0.01708 B^2 - 0.57583 C^2 - 0.08708 D^2

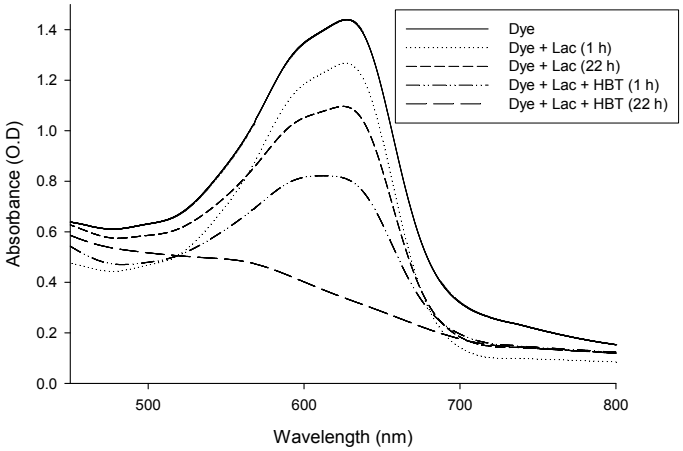


Fig. 1. Effect of HBT-laccase mediator system on Cibacron Blue 3G-A decolorization

Dye concentration, 100 ppm; laccase enzyme, 0.5 U/mL; HBT (in case of using HBT), 1 mM; pH: 4.5 (0.1 M citrate buffer); contact time, as indicated and temperature, 30ºC

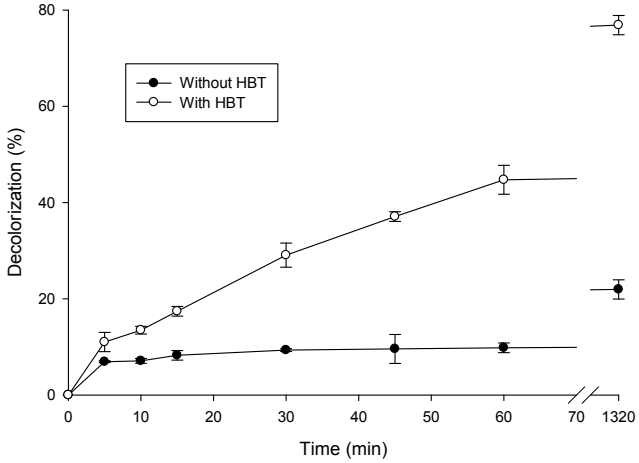


Fig. 2. Effect of HBT-laccase mediator system on Cibacron Blue 3G-A decolorization as a function of time

Dye concentration, 100 ppm; enzyme, 0.5 U/mL; HBT (in case of using HBT), 1 mM; pH: 4.5 (0.1 M citrate buffer); contact time, as indicated and temperature, 30ºC

Table 2. The coded and actual values of used variables of central composite design runs and the actual, predicted response and R-studentized residual values of Cibacron Blue 3G-A decolorization percentage

Run	Coded values				Actual values				Response (Decolorization, %)		R-studentized residual
	A (Laccase activity)	B (Dye conc.)	C (Time)	D (HBT)	Laccase activity (U/mL)	Dye conc. (ppm)	Time (min)	HBT (mM)	Actual value	Predicted value	
1	1	-1	-1	-1	0.500	50	15	0.50	14.34 ± 0.09	17.87	-3.53
2	-1	-1	1	1	0.250	50	35	1.00	26.72 ± 1.22	26.46	0.26
3	1	-1	1	-1	0.500	50	35	0.50	27.95 ± 0.006	26.97	0.98
4	0	0	0	0	0.375	75	25	0.75	24.85 ± 2.01	23.84	1.01
5	-1	1	-1	-1	0.250	100	15	0.50	15.59 ± 1.08	15.87	-0.28
6	-1	1	1	-1	0.250	100	35	0.50	15.5 ± 0.00	19.97	-4.47
7	-1	-1	1	-1	0.250	50	35	0.50	26.61 ± 0.24	25.18	1.43
8	0	0	-2	0	0.375	75	5	0.75	14.93 ± 0.97	15.55	-0.62
9	1	1	-1	-1	0.500	100	15	0.50	19.7 ± 1.33	18.68	1.02
10	0	0	0	0	0.375	75	25	0.75	24.84 ± 0.00	23.84	1.00
11	1	1	-1	1	0.500	100	15	1.00	20.09 ± 0.47	22.68	-2.59
12	-1	1	1	1	0.250	100	35	1.00	25.63 ± 0.21	23.26	2.37
13		1	1	-1	0.500	100	35	0.50	27.16 ± 0.03	27.09	0.07
14	0	0	0	0	0.375	75	25	0.75	25.21 ± 0.45	23.84	1.37
15	-1	1	-1	1	0.250	100	15	1.00	20.7 ± 2.42	20.40	0.3
16	0	0	0	-2	0.375	75	25	0.25	22.34 ± 0.00	20.85	1.49
17	1	-1	-1	1	0.500	50	15	1.00	25.61 ± 0.39	19.86	5.75
18	0	0	0	0	0.375	75	25	0.75	26.16 ± 1.85	23.84	2.32
19	2	0	0	0	0.625	75	25	0.75	24.49 ± 0.32	24.52	-0.03
20	1	-1	1	1	0.500	50	35	1.00	26.83 ± 0.06	27.71	-0.88
21	0	0	0	2	0.375	75	25	1.25	24.51 ± 0.13	26.13	-1.62
22	0	0	0	0	0.375	75	25	0.75	22.29 ± 0.00	23.84	-1.55
23	0	2	0	0	0.375	125	25	0.75	24.74 ± 0.35	22.57	2.17
24	0	-2	0	0	0.375	25	25	0.75	22.67 ± 0.18	24.96	-2.29
25	1	1	1	1	0.500	100	35	1.00	29.19 ± 2.06	29.83	-0.64
26	0	0	2	0	0.375	75	45	0.75	28.01 ± 1.82	27.51	0.5
27	-1	-1	-1	-1	0.250	50	15	0.50	22.29 ± 2.14	20.38	1.91
28	0	0	0	0	0.375	75	25	0.75	19.66 ± 0.00	23.84	-4.18
29	-2	0	0	0	0.125	75	25	0.75	20.37 ± 1.63	20.46	-0.09
30	-1	-1	-1	1	0.250	50	15	1.00	21.69 ± 0.03	22.91	-1.22

The analysis of variance (ANOVA) outcomes for the previous equation are existed in Table 3 which declare that the F-value of model that equal 2.85 means the significance of the model and there is just a 2.65% possibility that a "Model F-Value" this large could happen because of noise. As a base, "Prob > F" values less than 0.05 signify the significance of model terms whereas values bigger than 0.1000 point to the non significance of those model terms. From that, C (Time) and D (HBT) are significant in the current model where they have "Prob > F" values of 0.0002 and 0.047, respectively. In this connection, the "Lack of Fit F-value" of 1.78 means that the Lack of Fit is not significant comparative to the pure error and the insignificance of lack of fit is desired as the model should fit. There is a 27.20% probability that a "Lack of Fit F-value" could take place as a result of noise. "Adeq Precision" is a term used to evaluate the signal in relation to noise ratio, where the ratio bigger than 4 is wanted. The obtained ratio from the current model with a value of 6.779 shows a satisfactory signal.

3.3 Optimization of Cibacron Blue 3G-A Decolorization using RSM

The optimum conditions for Cibacron Blue 3G-A decolorization were found to be under using 0.50 U/mL of laccase, 92.19 ppm of Cibacron Blue 3G-A, and 1 mM of HBT in order to get decolorization percentage of 29.29% in 35 min.

The effects of studied parameters on Cibacron Blue 3G-A decolorization were revealed in the course of Figs. 3–8. The effect of laccase activity and Cibacron Blue 3G-A concentration on the response (Decolorization percentage) at fixed values of HBT concentration (0.75 mM), and reaction time (25 min) is exposed in Fig. 3. Increase in laccase activity and decrease in Cibacron Blue 3G-A concentration leads to an increase in Cibacron Blue 3G-A decolorization.

Fig. 4. Represents the contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of laccase activity and reaction time which indicates that the increase in both laccase activity and reaction time yielded a higher decolorization percentage. The highest response was obtained at 35 min and 0.5 U/mL of laccase activity.

The effect of laccase activity and HBT concentration as a mediator on Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase was illustrated in Fig. 5. The resulted surface response indicates that Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase is directly proportional to the increase in both of laccase activity and HBT concentration to attain maximum response at 0.5 U/mL of laccase and 1 mM of HBT as a mediator.

The contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of dye concentration and reaction time was shown in Fig. 6. The results obtained indicated that dye decolorization was increased by the gradual increase in reaction time and a decrease in dye concentration.

The effect of dye concentration and HBT concentration was represented in Fig. 7, where the contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase indicated that the increase in HBT concentration leads to an increase in the resulted response. In opposite direction the decolorization percentage was increased by the decrease in dye concentration.

The response (Cibacron Blue 3G-A decolorization) dependence on reaction time and HBT concentration was illustrated in Fig. 8. The obtained results declared that the decolorization percentage was increased by the increase in both reaction time and HBT concentration with maximum decolorization percentage at 1 mM of HBT after 35 min of reaction.

It is imperative to evaluate the fitted model to guarantee that it provide adequate approximation of the results achieved under the experimental conditions. By drawing a normal probability plot of the residuals, we could test out the normality assumption as presented in Fig. 9. For satisfaction of the normality assumption, the residuals plot should be approximated in a straight line. On the other hand, the R^2 (Coefficient of multiple regression), is a comprehensive statistic factor to evaluate the model fit. In the current model case, R^2 was found to be 0.7267 which signify the model suitability. For additional model justification, the calculated externally studentized residuals were presented in Table 2, where all the R-studentized residual values are positioned in a narrow difference range between the actual and predicted values which certify the model.

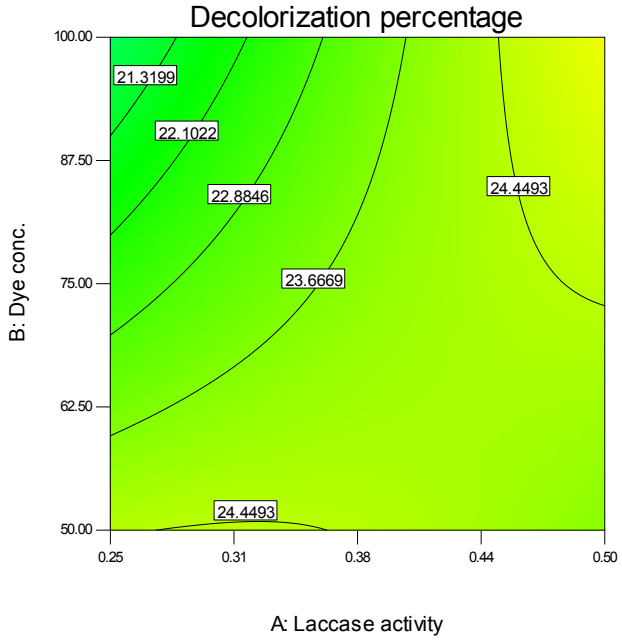


Fig. 3. Contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of laccase activity (U/mL) and dye concentration (ppm) at fixed values of HBT concentration (0.75 mM), and reaction time (25 min)

Table 3. ANOVA analysis for response surface quadratic model

Source	Sum of squares	Degree of freedom	Mean square	F-Value	Prob > F
Model	353.91	14	25.28	2.85	0.027
A-Laccase activity	24.77	1	24.77	2.79	0.115
B-Dye conc.	8.57	1	8.57	0.967	0.341
C-Time	214.44	1	214.44	24.18	0.0002
D-HBT	41.76	1	41.76	4.71	0.047
AB	28.36	1	28.36	3.20	0.094
AC	18.49	1	18.49	2.08	0.169
AD	0.297	1	0.297	0.033	0.857
BC	0.483	1	0.483	0.054	0.819
BD	4.00	1	4.00	0.451	0.512
CD	1.58	1	1.58	0.178	0.679
A^2	3.094	1	3.094	0.349	0.564
B^2	0.008	1	0.008	0.0009	0.976
C^2	9.095	1	9.095	1.02	0.327
D^2	0.208	1	0.208	0.023	0.880
Residual	133.04	15	8.87		
Lack of Fit	103.89	10	10.39	1.782	0.272
Pure Error	29.15	5	5.83		
Cor Total	486.95	29			

R-Squared, 0.7267; Adj R-Squared, 0.4717

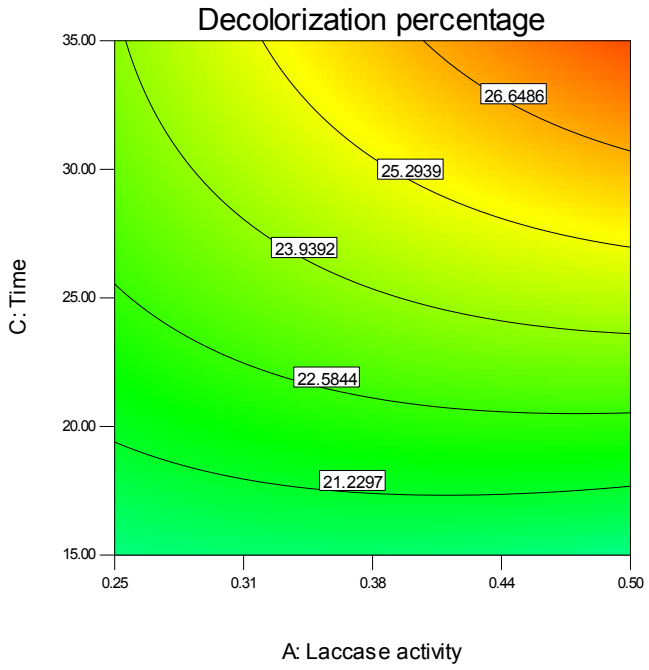


Fig. 4. Contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of laccase activity (U/mL) and reaction time (min) at fixed values of dye concentration (75 ppm) and HBT concentration (0.75 mM)

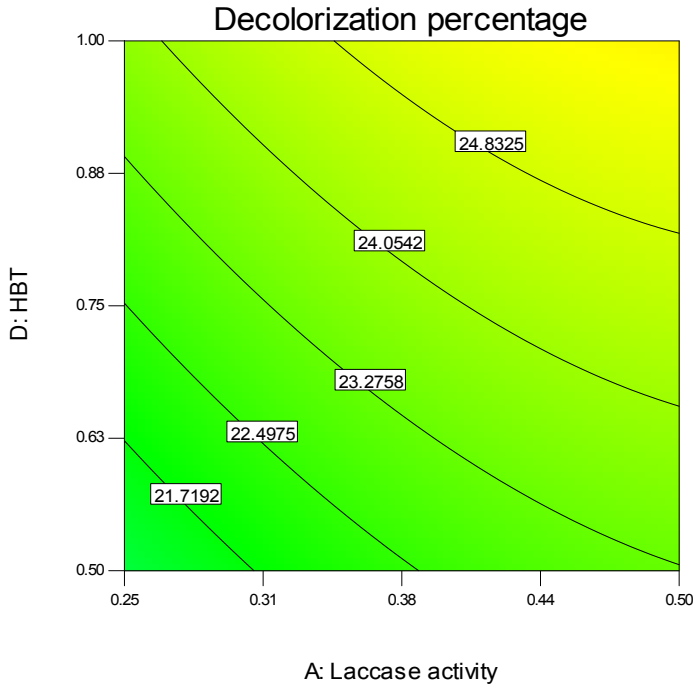


Fig. 5. Contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of laccase activity (U/mL) and HBT concentration (mM) at fixed values of reaction time (25 min) and dye concentration (75 ppm)

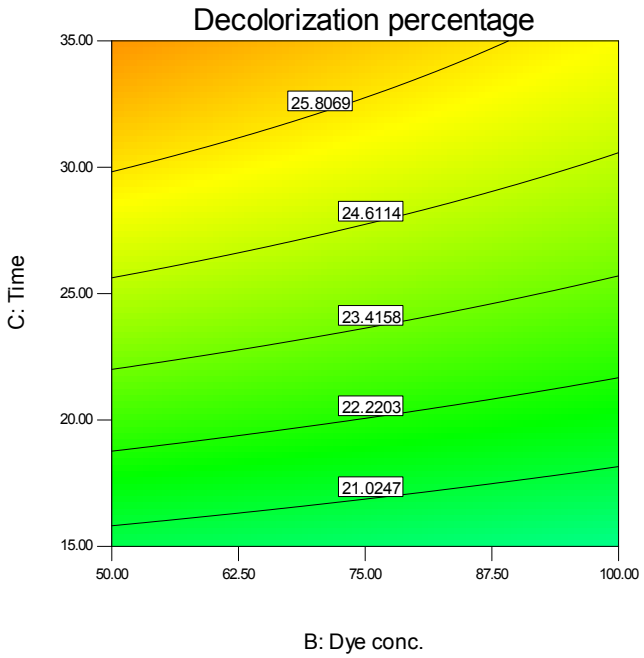


Fig. 6. Contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of dye concentration (ppm) and reaction time (min) at fixed values of laccase activity (0.38 U/mL) and HBT concentration (0.75 mM)

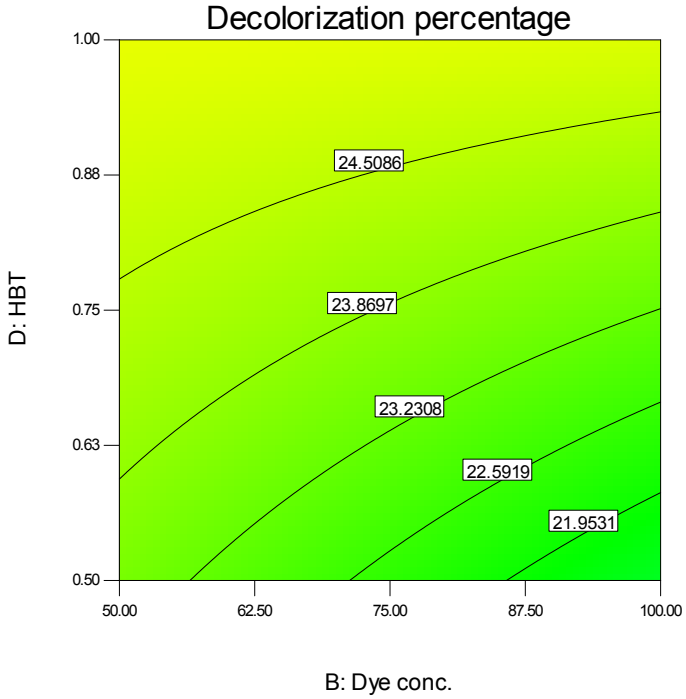


Fig. 7. Contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of dye concentration (ppm) and HBT concentration (mM) at fixed values of reaction time (25 min) and laccase activity (0.38 U/mL)

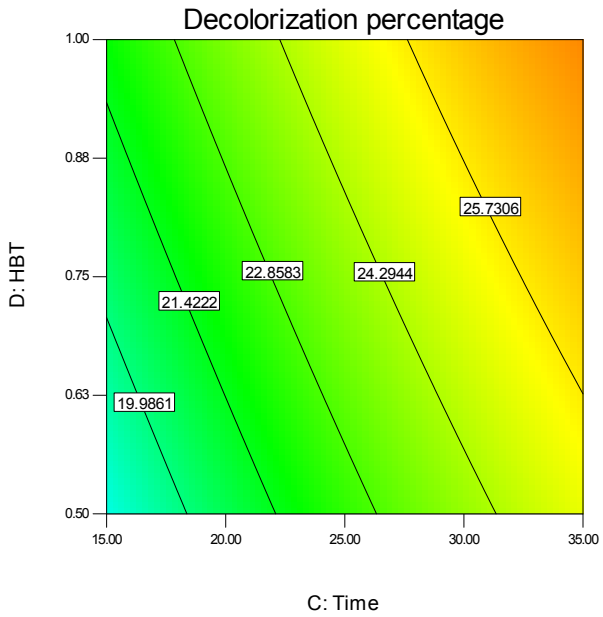


Fig. 8. Contour plot of Cibacron Blue 3G-A decolorization by *A. bisporus* CU13 crude laccase as a function of reaction time (min) and HBT concentration (mM) at fixed values of dye concentration (75 ppm) and laccase activity (0.38 U/mL)

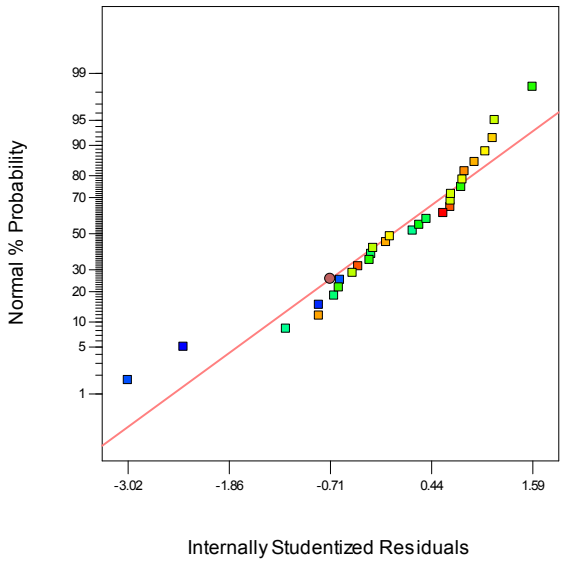


Fig. 9. The relation between internally studentized residuals and normal % probability

4. CONCLUSION

A. bisporus CU13 crude laccase was used as a biocatalyst to decolorize Cibacron Blue 3GA in presence of HBT as a mediator. The decolorization process was optimized through utilizing the response surface methodology approach. HBT affects the decolorization levels considerably. HBT concentration, dye concentration, enzyme activity, and incubation time were chosen as study variables to optimize Cibacron Blue 3G-A dye decolorization through CCD approach. The optimum conditions for Cibacron Blue 3G-A decolorization were found to be under using 0.50 U/mL of *A. bisporus* CU13 laccase, 92.19 ppm of Cibacron Blue 3G-A, and 1 mM of HBT in order to get decolorization percentage of 29.29% in 35 min.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Elshafei A, Othman A, Hassan M, Haroun B, Elsayed M, Farrag A. Catalyzed mediator-based decolorization of five synthetic dyes by *Pleurotus ostreatus* ARC280 Laccase. Br Biotechnol J. 2015;9(2):1–15.
2. Elshafei A, Elsayed M, Hassan M, Haroun B, Othman A, Farrag A. Biodecolorization of six synthetic dyes by *Pleurotus ostreatus* ARC280 laccase in presence and absence of hydroxybenzotriazole (HBT). Annu Res Rev Biol. 2017;15(4):1–16.
3. Slokar YM, Majcen Le Marechal A. Methods of decoloration of textile waste waters. Dyes Pigments. 1998;37(4):335–56.
4. Cristóvão RO, Tavares AP, Ribeiro AS, Loureiro JM, Boaventura RA, Macedo EA. Kinetic modelling and simulation of laccase catalyzed degradation of reactive textile dyes. Bioresour Technol. 2008;99(11): 4768–74.
5. Tavares APM, Cristóvão RO, Loureiro JM, Boaventura RAR, Macedo EA. Application of statistical experimental methodology to optimize reactive dye decolourization by commercial laccase. J Hazard Mater. 2009;162(2):1255–60.
6. Othman AM, Elshafei AM, Hassan MM, Haroun BM, Elsayed MA, Farrag AA. Purification, biochemical characterization and applications of *Pleurotus ostreatus* ARC280 Laccase. Br Microbiol Res J. 2014;4(12):1418-39.
7. Othman AM, Elsayed MA, Elshafei AM, Hassan MM. Purification and biochemical characterization of two isolated laccase isoforms from *Agaricus bisporus* CU13 and their potency in dye decolorization. Int J Biol Macromol. 2018;113:1142–8.
8. Othman AM, González-Domínguez E, Sanromán Á, Correa-Duarte M, Moldes D. Immobilization of laccase on functionalized multi walled carbon nanotube membranes and application for dye decolorization. RSC Adv. 2016;6(115):114690–7.
9. Wong Y, Yu J. Laccase-catalyzed decolorization of synthetic dyes. Water Res. 1999;33(16):3512–20.
10. Soares GMB, Amorim MTP essoa, Hrdina R, Costa-Ferreira M. Studies on the biotransformation of novel disazo dyes by laccase. Process Biochem. 2002;37(6): 581–7.
11. Gamelas JAF, Tavares APM, Evtuguin DV, Xavier AMB. Oxygen bleaching of kraft pulp with polyoxometalates and laccase applying a novel multi-stage process. J Mol Catal B Enzym. 2005;33(3):57–64.
12. Elsayed MA, Othman AM, Hassan MM, Elshafei AM. Optimization of silver nanoparticles biosynthesis mediated by *Aspergillus niger* NRC1731 through application of statistical methods: enhancement and characterization. 3 Biotech. 2018;8:132.
13. Othman AM, Elsayed MA, Elshafei AM, Hassan MM. Application of response surface methodology to optimize the extracellular fungal mediated nanosilver green synthesis. J Genet Eng Biotechnol. 2017;15(2):497–504.
14. Box GEP, Hunter WG, Hunter JS. Statistics for experimenters: An introduction to design, data analysis and model building. Wiley. 1978;678.
15. Tavares APM, Cristóvão RO, Loureiro JM, Boaventura RAR, Macedo EA. Application of statistical experimental methodology to optimize reactive dye decolourization by commercial laccase. J Hazard Mater. 2009;162(2-3):1255–60.
16. Smith WO, Daniels SM. Purification of Phytochrome by Affinity Chromatography on Agarose-Immobilized Cibacron Blue 3GA. Plant Physiol. 1981;68(2):443–6.
17. von Kügelgen I, Späth L, Starke K. Evidence for P2-purinoceptor-mediated inhibition of noradrenaline release in rat brain cortex. Br J Pharmacol. 1994; 113(3):815–22.
18. Bennett GC, Boarder MR. The effect of nucleotides and adenosine on stimulus-evoked glutamate release from rat brain cortical slices. Br J Pharmacol. 2000; 131(3):617–23.
19. Schneider KD, Bethel CR, Distler AM, Hujer AM, Bonomo RA, Leonard DA. Mutation of the active site carboxy-

lysine (K70) of OXA-1 beta-lactamase results in a deacylation-deficient enzyme. Bio-chemistry (Mosc). 2009;48(26):6136–45.

20. Othman AM, Elsayed MA, Elshafei AM, Hassan MM. Application of central composite design as a strategy to maximize the productivity of *Agaricus bisporus* CU13 laccase and its application in dye decolorization. Biocatal Agric Biotechnol. 2018;14:72–9.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72:248–54.

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