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Optimization of dye removal from aqueous Remazol Brilliant Blue R (RBBR) by *Trametessp*. Pellets

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ABSTRACT

Decolorization of Remazol Brilliant Blue R (RBBR) by *Trametessp*. Pellets was studied in a batch reactor. Dye removal process was performed in shaken flasks which contained 100 ml of RBBR aqueous solution and fungal pellets. The process was followed for 48 h and the dye removal was analyzed at a visible spectrum of 590 nm. Response surface methodology (RSM) employing Box Behnken design at three factors i.e. initial concentration of RBBR, mass of pellet and pH was used to optimize the decolorization process) with three replicates. Response surface regression showed that the decolorization efficiency was affected by initial RBBR concentration. Mass of pellet and pH in this model were not found to be insignificant for both main and square effects. The dye decolorization varied within the range of 16.81% to 77.91%. The lowest decolorization was achieved in maximum initial concentration dye and pH. While the highest decolorization was observed when low initial dye concentration and pH were used. From the optimization, maximum dye removal efficiency of 67.9% ± 5.43 was achieved at 50 ppm RBBR solution, 4 gram of pellets at pH 5.6.

1. Introduction

Approximately 10,000 different dyes and pigments are manufactured worldwide with the total market of more than 7 x 105tonnes per year. There are several structural varieties of dyes, such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based, and metal complex dyes (Doble and Kumar 2005). They are used as coloring agents in food, cosmetics, paper, plastic, and textile industries (Cetin and Donmez 2006; Sathiya et al. 2007). Two percent of dyes that are produced by dye industries are discharged directly in the effluent. In addition, 10% is lost during coloration process in textile manufacture (Doble and Kruthiventy 2007). Reactive dyes are widely used in textile industries to colour the cellulosic fibres (Tavares et al. 2009). More than 80,000 tonnes of reactive dyes are produced and consumed every year. This condition described the total amount of pollution generated (Hessel et al. 2007). Studies have shown that many of the dyes are carcinogenic, mutagenic, and highly harmful to the environment. Untreated dye effluent is highly colored and hence may significantly affect photosynthesis activity due to reduced light penetration (Banat et al. 1996). Several technologies are available for decolorization of textile dye effluents, such as adsorption, irradiation, ion exchange, oxidation, coagulation and precipitation, aerobic process, and anaerobic process. But the problem has not been solved because of high cost, low efficiency, sludge handling problems, less microbial resistant to the pollutant, etc. (Anjaneyulu et al. 2005). Recently, many studies of biological decolorization utilizing fungal strains have been reported (Deveci et al. 2004). White rot fungi have been shown to degrade a wide variety of recalcitrant organic pollutant (Young and Yu 2007). The several fungal strains were found potential to decolorize commercial reactive dyes e.g. Bjerkanderaadusta, Trametesversicolor and Phanerochaetechrysos-porium (Heinflinget al. 1997; Swamy and Ramsay 1999).

In this study, *Trametessp* pellets were used for decolorizing reactive dye Remazol Brilliant Blue R (RBBR). RBBR is usually used in the production of polymeric dyes. This dye is an anthraquinone derivative which represents a class of toxic and organopollutant materials (Wesenberg et al. 2003). The aim of the studies was to investigate the effect of initial dye concentration, pH, and mass of fungal pellet to decolorization, and optimizing all the variables using response surface methodology to reach the maximum decolorization.

2. Materials and methods

2.1. Dye

Reactive Remazol Brilliant Blue R (RBBR) was purchased from Sigma Aldrich, Inc. This dye also has known as Cavalite Brilliant Blue R, C.I. Reactive Blue 19, C.I. Reactive Blue 19 disodium salt, Reactive Blue 19, or Remalan Brilliant Blue R with the empirical formula C22H16N2Na2O11S3 and the molecular weight is 626.54. The RBBR was classified as Vinyl Sulfone Reactive dye. Chemical formula of RBBR is presented in Fig.1.

2.2. Culture

Trametessp culture was obtained from the Institute of Biological Sciences University of Malaya. The white rot fungus was cultivated on agar medium containing (per liter) 20 g glucose, 0.5 g $MgSO_4.7H_2O$, 1 g K_2HPO_4, 0.46 g KH_2PO_4, 2 g yeast extract, 2 g malt extract, 2 g peptone, 0.1 g NH_4Cl and 18 g agar, and incubated at 28 ^0C for 7 days.

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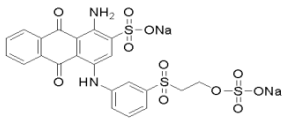
Chemical Formula	
Synonyms	Cavalite Brilliant Blue R, C.I. Reactive Blue 19, C.I. Reactive Blue 19 disodium salt, Reactive Blue 19, Remalan Brilliant Blue R
Empirical formula	$C_{22}H_{16}N_2Na_2O_{11}S_3$
Molecular weight	626.54
Classification	Vinyl Sulfone Reactive Dye

Fig. 1. Remazol Brilliant Blue R.

2.3. Fungal pellet

Fungal pellets were grown in 100 ml liquid medium in 250 ml flask. Culture medium for growing the pellet contained (g/L); 20 glucose, 0.5 $MgSO_4.7H_2O$, 1 K_2HPO_4, 0.46 KH_2PO_4, 2 yeast extract, 2 malt extract, and 2 peptone. This medium was aseptically inoculated with 5 ml mycelium suspension from actively growing culture on agar plate. Subsequently, this culture was incubated on an orbital shaker (160 rpm) at 28^0C for 5 days.

2.4. Response surface design

In this study, response surface employing Box-Behnken design at three factors (initial dye concentration, pH, and mass of pellet) with three replicates was employed. These factors were selected because they were hypothesized to affect decolorization reaction. The levels of the factors are presented in Table 1 and total experimental runs are given in Table 2.

Table 1. Experimental factor and their levels for a three-level Box-Behnken design.

Coded factor	Factors	Coded level		
		-1	0	+1
x_1	Initial dye concentration	50	65	80
x_2	mass of pellet	4	5	6
x_3	pH	4	5	6

The experimental design and analysis of variance (ANOVA) were performed using Minitab® Release 14.12.0 statistical software (Minitab Inc.). A polynomial regression model was used to approximate the response (eq.1).

$$\eta = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

where η is the response (decolorization efficiency); X_1, X_2, X_3 are coded levels of the independent factors.

Table 2. Experimental data for RBBR decolorization and percentage of dye removal.

Run	Factors			% decolorization		Run	Factors			% decolorization	
	x_1(ppm)	x_2	x_3(g)	Actual	Predicted		x_1(ppm)	x_2	x_3(g)	Actual	Predicted
1	65	6	6	27.96	29.19	24	65	6	6	25.26	29.19
2	80	5	6	20.40	21.75	25	50	4	5	68.73	63.42
3	50	5	4	59.20	61.78	26	65	4	4	23.47	30.86
4	50	4	5	72.11	63.42	27	65	5	5	30.22	29.22
5	50	5	6	47.45	61.78	28	65	5	5	30.32	29.22
6	65	5	5	29.61	29.22	29	65	6	6	28.66	29.19
7	80	5	6	18.76	21.75	30	80	4	5	20.95	23.39
8	65	6	4	28.59	29.19	31	65	4	6	34.84	30.86
9	80	6	5	21.87	21.72	32	65	5	5	32.26	29.22
10	65	4	4	22.89	30.86	33	50	5	6	53.83	61.78
11	65	6	4	33.30	29.19	34	50	6	5	61.43	61.75
12	65	5	5	30.90	29.22	35	80	5	6	16.81	21.75
13	80	5	4	26.51	21.75	36	50	5	4	77.91	61.78
14	50	5	6	55.94	61.78	37	50	4	5	62.44	63.42
15	80	5	4	27.47	21.75	38	65	4	6	35.77	30.86
16	65	6	4	24.36	29.19	39	65	5	5	30.80	29.22
17	50	5	4	55.98	61.78	40	65	5	5	31.18	29.22
18	50	6	5	71.66	61.75	41	65	5	5	30.79	29.22
19	65	5	5	30.55	29.22	42	65	4	6	32.65	30.86
20	80	6	5	20.22	21.72	43	80	4	5	22.03	23.39
21	65	4	4	28.98	30.86	44	80	5	4	27.29	21.75
22	80	6	5	22.76	21.72	45	80	4	5	20.82	23.39
23	50	6	5	59.57	61.75						

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The regression coefficients are: β_0 the constant term; β_1, β_2, β_3 the coefficients for linear effects; β_{11}, β_{22}, β_{33} the coefficients for quadratic effects, and β_{12}, β_{13}, β_{23} the coefficient for interaction effects.

2.5. Decolorization studies

The stock solution of RBBR was prepared at 250 ppm and required concentrations were obtained by dilutions. Dye decolorization was performed in Erlenmeyer flasks using the 5-day old pellets at various levels of pH, mass of pellets and initial dye concentration as formulated by the experimental design. The initial pH of aqueous RBBR solution valued 5.6 was adjusted to the desired value using 0.01 M HCl and 0.01 M NaOH.

The flasks were shaken at 160 rpm for 2 days. Samples were withdrawn from the flask, and centrifuged at 7000 rpm for 15 minutes. The absorbance of supernatant was analyzed at a visible spectrum of 590 nm using UV-Vis spectrophotometer (Jasco, Japan). A standard calibration curve was prepared for the dye and the equation is:

$$C_{dye} = 101.83Abs \quad (2)$$

where C dye is concentration of dye (ppm), and Abs is absorbance. The coefficient of correlation (R^2) value for above equation is 0.99143. The amount of decolorization was determined using eq. (3).

$$Decolorization(\%) = \frac{(C_i - C_f)}{C_i} \times 100\% \quad (3)$$

where C_i is the initial concentration of dye (ppm) and C_f is the final concentration (ppm).

3. Results and discussion

After The results of 45 run for three variables (initial dye concentration, pH, and mass of pellets) for RBBR decolorization are shown in Table 2. The dye decolorization varied within the range of 16.81% to 77.91%. The lowest decolorization was achieved in maximum initial concentration dye and pH. While the highest decolorization was observed when low initial dye concentration and pH were used. Response surface methodology (RSM) was employed to identify the factors that influenced decolorization process, to evaluate interaction that may exist between the factors, and to optimize the factors level to get the maximum decolorization.

A p value is the indicator of the significance of the test, a value below 0.05 indicates that test parameter is significant at 5% level of significance. Analysis of variance (ANOVA) for percentage decolorization which is presented by Table 3 showed that the polynomial regression model is not a fit regression for the data, where the p value of lack of fit is 0.000. It indicates the significant value for lack of fit. Main and square effects are found to be significant ($p < 0.05$), and interaction effects are not significant at 5% level.

Getting a fit regression model, a best subset regression could be employed to find the selection variables for model building as illustrated by Table 4. A good model shouldhave high R^2 and adjusted R^2, small *S*, and Mallows' *Cp* close to the number of variables plus the constant contained in the model. From the Table 4, the Mallows *Cp* value is closed to row 4 (initial concentration of dye and pH), with R^2 (76.5) and adjusted R^2 (75.4).

To verify the model, the response surface analysis is operated to the selected variables using the linear and square regression model. A new analysis of variance from this model was presented in Table 5. ANOVA shows that fitted linear and square model is highly significant with F-test = 95.08 (p = 0.000), and not significant for the lack of fit (p = 0.147).

Table 3. Analysis of variance for decolorization by polynomial regression model.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	11619.1	11619.1	1291.01	46.89	0.000
Linear	3	9690.1	9690.1	3230.04	117.31	0.000
Square	3	1821.5	1821.5	607.15	22.05	0.000
Interaction	3	107.5	107.5	35.85	1.30	0.289
Residual Error	35	963.7	963.7	27.53		
Lack of fit	3	421.6	421.6	140.53	8.30	0.000
Pure error	32	542.1	542.1	16.94		
Total	44	12582.8				

Table 4. Best subsets regression: percentage of decolorization versus concentration of dye, mass of pellets.

No.	R^2	R^2 (adj)	Mallows *Cp*	S	Initial conc.	Pellets mass	pH
1	76.4	75.9	1.1	8.3086	X		
1	0.5	0.0	136.5	17.066		X	
2	76.9	75.8	2.2	8.3230	X	X	
2	76.5	75.4	2.8	8.3832	X		X
3	77	75.3	4	8.3996	X	X	X

The regression coefficients, t and p values for all the linear, quadratic effects of the variables are given in Table 6. A reduced polynomial was generated relating the response to the significant linear and square regression model i.e:

$$\eta = 29.22 - 20.015X_1 - 0.835X_2 + 12.5467X_1^2 + 0.8117X_2^2 \quad (4)$$

Table 5. Analysis of variance for decolorization using the linear and square regression model.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	11385.3	11385.31	2846.33	95.08	0.000
Linear	2	9631.1	9631.14	4815.57	160.85	0.000
Square	2	1754.2	1754.18	877.09	29.30	0.000
Residual Error	40	1197.5	1197.50	29.94		
Lack of fit	4	201.2	201.26	50.29	1.82	0.147
Pure error	36	996.3	996.34	27.68		
Total	44	12582.8				

Table 6. Estimated regression coefficients for percentage decolorization of RBBR.

Terms	Coefficients	SE coefficients	t-value	p-value
Constant	29.22	1.518	19.25	0.000
dye concentration	-20.015	1.117	-17.92	0.000
pH	-0.835	1.117	-0.748	0.459
dye concentration*dye concentration	12.5467	1.639	7.655	0.000
pH*pH	0.8117	1.639	0.495	0.623

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The coefficient of correlation R^2 for the above equation was 90.48%. Therefore, this equation can be used for predicting response at any combination of three variables within experimental range. The closer the values of R to 1, better the correlation between the experimental and predicted values (Montgomery, 2005). The predicted response value using eq. (4) are shown in Table 2. For the main factors, only initial dye concentration is found to be significant ($p< 0.05$), where the decolorization percentage decreasing with the initial dye concentration (Fig. 2).

The pH ($p = 0.459$) is found to be insignificant on dye decolorization within the experimental range tested. Meanwhile, for the square effect only initial dye concentration is found to be significant to the decolorization percentage ($p<0.05$). The square effect of pH ($p = 0.623$) is insignificant on the decolorization of dye.

Response Optimizer function of Minitab was used to find the optimum values of key variables. The solution of eq. (4) is aimed to result in minimum 65% of the dye decolorization. Based on this, a global solution of 50 ppm initial concentration of dye, pH 4 are obtained from the initial point of 50 ppm initial dye concentration, 4 gram pellets mass at pH 4. The maximum percentage of decolorization predicted by the software was 63.43%, with composite desirability value of 0.96. For practical purposes, the value of the pH is kept at 5.6, respectively in view of the scaling up process.

The slight difference in the value of pH and mass of the pellet was proven to be insignificant to the degree of decolorization as shown by the simulation results where percentage of decolorization was predicted at 61.57%, with composite desirability value of 0.90. Verification experiments were subsequently performed to confirm the optimization results. The decolorization percentage from these experiments was 67.9% ± 5.4.

4. Conclusions

The aqueous RBBR decolorization studies were successfully optimized by response surface methodology. Response surface method explained the effect of the factors and the possible interactions.

The linear and square regression model showed a good fit to the experimental data (R^2= 90.48%). From the experiment, initial dye concentration was found to be a significant main factor to decolorization process. After optimization by using statistical analysis, a verification experiment was done and the maximum decolorization efficiency of 67.9 ± 5.4% was achieved when optimum value of 50 ppm initial concentration of dye, 4 grams of pellet and pH 5.6 were used.

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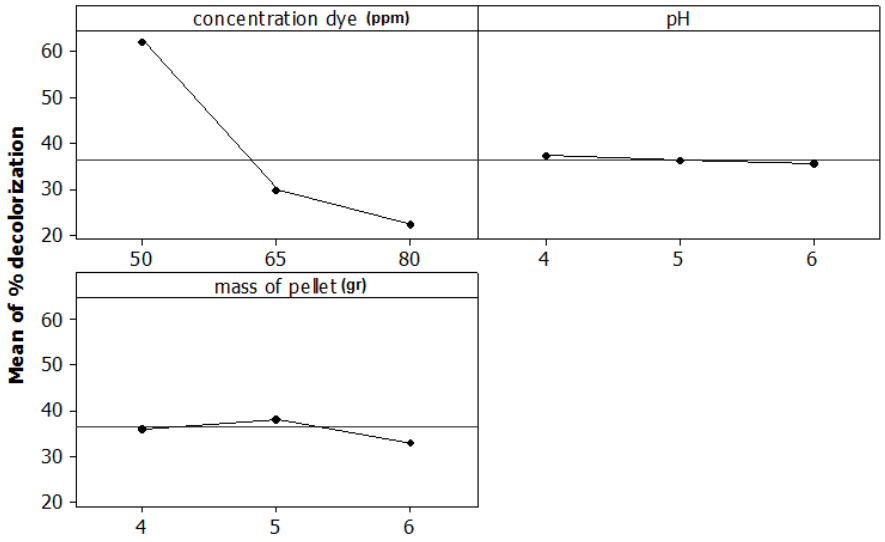

Fig. 2. Main effects plot (data means) for percentage of decolorization.

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