

Textile dye wastewater treatment using *coriolus versicolor*

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Abstract. Decolourization potential of white rot fungal organism, *coriolus versicolor*, was investigated in a batch reactor, for textile dye industry wastewater. The influence of process parameters like pH, temperature, agitation speed and dye wastewater concentration on the decolourization of textile dye wastewater was examined by using Response surface methodology (RSM). The maximum decolourization was attained at: pH- 6.8, temperature – 27.9°C, agitation speed – 160 rpm and dye wastewater concentration – 1:2. From the analysis of variance (ANOVA) results it was found that, the linear effect of agitation speed and dye wastewater concentration were significant for the decolourization of textile dye wastewater. At these optimized condition, the maximum decolourization and chemical oxygen demand (COD) reduction was found to be 64.4% and 79.8% respectively. Various external carbon sources were tried to enhance the decolourization of textile dye wastewater. It was observed that the addition of carbon source enhances the decolourization of textile dye wastewater. Kinetics of textile dye degradation process was studied by first order and diffusional model. From the results it was found that the degradation follows first order model with R² value of 0.9430.

Keywords: decolourization; RSM; *coriolus versicolor*; optimization; kinetics; COD

1. Introduction

Textile dye industry wastewater is one of the complex wastewater due to its colour, high toxicity, biological oxygen demand (BOD), chemical oxygen demand (COD), turbidity, total dissolved solids (TDS), total suspended solids (TSS), etc. Synthetic dyes used in textile industry contain chemicals of strong aromatic structure and possess highly stable and difficult to degradation (Kaushik and Malik 2009). Annually around 0.7 to 0.8 million tons of synthetic dyes are produced and roughly 10,000 different dyes are used industrially (Park *et al.* 2007). Due to lack of utilization on dyeing process up to 15% of dye was mixed with the process water (Sharma and Sobti 2000, Elisangela *et al.* 2009). All these dyes are resistant to fading by sweat, light, water and chemicals including oxidizing agent (Wong and Yu 1999). It affects the aquatic life by reducing photosynthesis due to coloration of water (Malik and Taneja 1994).

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Various physiochemical processes like adsorption, chemical oxidation and reduction; flocculation, photolysis, etc. have been applied for the removal of color and pollutants present in textile dye wastewater (Zhang *et al.* 2002, Rajeshkannan *et al.* 2010, 2011). Though physico-chemical treatments are effective for dye decolorization, they require more energy and chemicals. These processes concentrate the pollution into liquids or solids which require further treatment. Brightly colored, water-soluble reactive and acid dyes present in dye wastewater tend to pass through conventional treatment systems unaffected (Shaul *et al.* 1991). Alternatively metabolic potential of microbial sources has received great attention in recent years for its efficiency and inexpensive. Hence biotechnological process is considered as an effective process in the treatment of dye wastewater with effective manner (Willmott *et al.* 1998). Microorganisms like bacteria, fungi and yeast are widely used for the decolorization of dye wastewater (Watanabe 2001). Among these, white rot fungi are capable of degrading synthetic dyes that are able to depolymerise and mineralize lignin because of their low substrate specificity and degrade the wide range of xenobiotic compounds.

Response surface methodology (RSM) is a combination of mathematical and statistical techniques used for developing, improving and optimizing the processes and used to evaluate the relative significance of several affecting factors even in the presence of complex interactions. RSM is widely used in enzyme production, food technology, environmental biotechnology etc. (Dilipkumar *et al.* 2010, Manivannan and Rajasimman 2011, Rajeshkannan *et al.* 2011). RSM is also used for the optimization of process conditions (Rajasimman *et al.* 2010). In this study, a white rot fungal strain, *coriolus versicolor* was used to treat the textile dye industry wastewater. In the decolorization process by fungi, there are various influencing factors. They can be grouped into two kinds: one is related to fungal growth conditions; the other is related to the characteristics of the dye solution or wastewater. As different components possess different abilities to decolorize dyes, it is necessary to create an optimal environment favorable to fungal growth and thus make the fungi possess the maximum ability to decolorize dyes in wastewater. Important fungal growth conditions are pH, temperature, oxygen, incubation time, nutrients, etc. (Fu and Viraraghavan 2001). Hence in this work, the process variables, pH, temperature, agitation time and dye wastewater concentration were selected and they were optimized using Response surface methodology (RSM).

2. Materials and methods

The textile dye wastewater was collected from a private small scale industry located at Erode, Tamilnadu, India. The characteristic of textile dye wastewater was analyzed as per the procedure given in American Public Health Association (APHA) and it was given in Table 1. The wastewater was stored at $4 \pm 1^\circ\text{C}$ in air tight plastic containers. The fungal strain *coriolus versicolor* (MTCC - 138) was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. This strain was maintained at 4°C on yeast extract glucose agar medium. The primary inoculum of this fungal was grown in Erlenmeyer flasks. The media composition was: yeast extract - 5 g, glucose - 10 g, distilled water - 1 L, pH - 5.8, and temperature - 25°C for 10 days. These inoculums were subjected as a biological agent for decolorization of dye industry effluent treatment. The subculture was routinely prepared at regular intervals of 30 days.

Table 1 Characteristics of textile dye industry wastewater

Parameters*	Values
pH	7.7-7.9
Colour	Brown
Total Suspended Solids	500-515
Total Dissolved Solids	3800-3925
BOD	1120-1185
COD	2410-2495

* All values except pH and colour are in mg/L

2.1 Response surface methodology (RSM)

In this work, Box-Behnken design was used to study the effects of the variables towards their responses and for optimization studies. This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments and the interaction between the parameters. A regression design is normally employed to model a response as a mathematical function of a few continuous factors and good model parameter estimates are desired.

The coded values of the process parameters are determined by the following equation

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

where x_i – coded value of the i^{th} variable, X_i – uncoded value of the i^{th} test variable and X_0 – uncoded value of the i^{th} test variable at center point.

The regression analysis was performed to estimate the response function as a second order polynomial

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1, i < j=2}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response, β_i , β_j , β_{ij} are coefficients estimated from regression. They represent the linear, quadratic and cross products of x_1 , x_2 , x_3 on response.

The regression and graphical analysis with statistical significance were carried out using Design-Expert software (version 7.1.5, Stat-Ease, Inc., Minneapolis, USA). In order to visualize the relationship between the experimental variables and responses, the response surface and contour plots were generated from the models. The adequacy of the models was further justified through analysis of variance (ANOVA).

2.2 Decolourization experiments

The range and levels of process parameters, pH, temperature, agitation speed and dye wastewater concentration were given in Table 2. In Erlenmeyer flask, 150 ml of textile dye wastewater (raw,

Table 2 Levels of different process variables in coded and un-coded form for the decolourization of textile dye industry wastewater

Variables	Code	Levels		
		-1	0	+1
pH	A	5	7	9
Temperature, °C	B	23	28	33
Agitation speed, rpm	C	100	150	200
Dye wastewater concentration	D	Raw	1:1	1:2

1:1, 1:2) was taken according to the Box-Behnken design given in Table 3. pH was adjusted (5, 7, 9) using 0.1 N HCl and 0.1 N NaOH followed by the addition of inoculums (3%) and kept at respective temperature (23, 28, 33°C) in an orbital shaker (REMI, India). Decolourization was monitored by UV spectroscopic analysis by changing in the absorbance of sample. Samples were withdrawn and centrifuged at 10,000 rpm for 10 mins to remove fungal mycelia. The pellet was discarded after centrifugation and clear solution was analyzed using the UV spectrophotometer (Model: BL-200, ELICO, India) at 395 nm. COD of the samples was analysed using the procedure given in American Public Health Association (APHA).

At the optimized condition, effect of external carbon source was studied by the addition of various carbon sources. 150 ml of 1:2 diluted wastewater was taken in various Erlenmeyer flasks. 10 g of sucrose, glucose, bagasse, pressmud, rice bran, wheat bran was added in separate flask and the decolourization was measured at regular intervals in UV spectrophotometer. The percentage decolourization and COD reduction are calculated using the following equations. The parameters like pH, temperature and agitation speed were maintained at the optimum condition.

$$\% \text{Decolourization} = \frac{\text{InitialOD} - \text{FinalOD}}{\text{InitialOD}} \times 100 \quad (3)$$

$$\% \text{COD}_{\text{reduction}} = \frac{\text{COD}_{\text{Initial}} - \text{COD}_{\text{Final}}}{\text{COD}_{\text{Initial}}} \times 100 \quad (4)$$

3. Results and discussion

The effect of process parameters, on the decolourization and degradation of textile dye wastewater were studied. The second order polynomial coefficients for each term of the equation (Eq. (5), (6)) were determined using the Design Expert 7.1.5. The experimental and predicted values of percentage decolourization and degradation were given in Table 3.

$$\% \text{Decolourization} = 57.50 - 1.22A - 3.35B - 0.32C + 8.88D - 3.30AB - 4.18AC - 0.075AD + 1.30BC + 2.95BD + 1.92CD - 5.37A^2 - 6.00B^2 - 4.15C^2 - 3.00D^2 \quad (5)$$

Table 3 Experimental conditions of Box Behnken design for textile dye wastewater degradation using *coriolus versicolor*

Run no.	pH	Temperature	Agitation speed	Dye wastewater concentration	%Decolourization		%Degradation	
					Experimental	Predicted	Experimental	Predicted
1	-1	0	0	1	61.9	59.31	77.5	68.85
2	1	-1	0	0	54.5	51.57	61.3	56.59
3	-1	0	0	-1	42.3	41.39	55.4	51.44
4	0	1	0	-1	30.5	33.33	42.3	42.75
5	0	0	1	-1	40.2	39.23	45.7	46.16
6	1	0	-1	0	48.9	51.26	52.1	54.24
7	0	0	-1	1	55.8	57.63	61.2	63.63
8	0	-1	0	1	60.5	57.79	65.4	65.30
9	0	-1	0	-1	45.3	45.93	51.2	50.84
10	0	-1	1	0	49.5	49.09	54.6	52.37
11	1	0	1	0	40.2	42.28	44.3	45.74
12	0	0	0	0	57.4	57.43	62.8	61.90
13	0	0	0	0	57.1	57.43	61.5	61.90
14	0	1	-1	0	43.6	43.03	48.3	47.29
15	1	0	0	1	56.8	56.73	62.5	63.22
16	0	1	0	1	57.5	56.99	61.5	62.22
17	1	1	0	0	41.3	38.27	46.6	41.61
18	1	0	0	-1	37.5	39.11	41.3	46.70
19	0	0	0	0	57.8	57.43	61.4	61.90
20	-1	-1	0	0	43.5	47.40	44.5	52.38
21	0	1	1	0	47.5	44.99	50.5	47.74
22	0	0	1	1	56.8	60.85	60.8	65.68
23	-1	0	1	0	55.3	53.06	60.7	58.92
24	0	-1	-1	0	50.8	52.33	54.3	53.82
25	0	0	-1	-1	46.9	43.72	51.2	49.21
26	-1	0	-1	0	47.3	45.34	52.5	51.42
27	-1	1	0	0	43.5	47.30	48.6	56.19
28	0	0	0	0	57.1	57.43	61.9	61.90
29	0	0	0	0	57.4	57.43	61.5	61.90
30	0	0	0	0	57.3	57.43	61.7	61.90

$$\% \text{COD reduction} = 61.66 - 2.59A - 2.79B - 0.25C + 8.48D - 4.70AB - 4.00AC - 0.22AD + 0.48BC + 1.25BD + 1.27CD - 3.85A^2 - 6.12B^2 - 5.23C^2 - 0.26D^2 \quad (6)$$

where A, B, C and D were the coded values of the process variables, pH, temperature, agitation speed and dye wastewater concentration respectively.

The results were analyzed by using Analysis of Variance (ANOVA) and were given in Table 4. The ANOVA of the quadratic regression model indicates the model is significant. In this work, the model F-value 12.58 and 4.68 for decolourization and COD reduction implies that the models were

Table 4 ANOVA for the decolourization and degradation of textile dye wastewater

Source	%Decolourization			%COD reduction		
	Coefficient factor	F value	P > F	Coefficient factor	F value	P > F
Model	57.50	12.58	<0.0001	61.66	4.63	0.0035
A	-1.22	1.88	0.1923	-2.59	3.24	0.0934
B	-3.35	14.23	0.0021	-2.79	3.76	0.0729
C	-0.32	0.13	0.7268	-0.25	0.030	0.8646
D	8.88	100.04	<0.0001	8.48	34.73	<0.0001
A*A	-5.37	19.77	0.0006	-3.85	3.86	0.0696
B*B	-6.00	24.63	0.0002	-6.12	9.77	0.0074
C*C	-4.15	11.78	0.0040	-5.23	7.14	0.0182
D*D	-3.00	6.15	0.0265	-0.26	0.018	0.8966
A*B	-3.30	4.0	0.0500	-4.70	3.55	0.0804
A*C	-4.18	7.37	0.0168	-4.00	2.57	0.1310
A*D	-0.075	0.00238	0.9618	-0.22	0.0081	0.9294
B*C	1.30	0.71	0.4123	0.48	0.036	0.8516
B*D	2.95	3.68	0.0758	1.25	0.25	0.6239
C*D	1.92	1.57	0.2313	1.27	0.26	0.6171
Lack of fit		176.30	<0.0001		80.00	0.0004

Table 5 Kinetic analysis of textile dye wastewater degradation by *coriolus versicolor*

Model	Rate Constant	Values	R ²
First order	k ₁	0.013	0.9430
Diffusional	k ₂	-0.212	0.9490

significant. The smaller the magnitude of the P , more significant is the corresponding coefficient. P value less than 0.05 indicate the model terms are significant. From the P values it is found that, the variables, B, D, AC, A², B², C², D² were significant model terms for decolourization and D, B², C² were significant model terms for COD reduction. From the ANOVA table it was found that the linear effect of dye wastewater concentration is more significant for textile dye wastewater treatment.

The predicted R^2 of 0.7660 (decolourization), 0.7405 (COD reduction) was in reasonable agreement with the adjusted R^2 of 0.8527 (decolourization) and 0.8447 (COD reduction). The fit of the model is also expressed by the coefficient of regression R^2 , which is found to be 0.9264 for decolourization and 0.9224 for COD reduction, indicating that more than 92% of the variability in the response could be explained by the model. This implies that the prediction of experimental data is quite satisfactory. The positive sign of coefficient factors in Table 4 indicates the positive contribution of pH, agitation speed and wastewater concentration and negative sign indicates the negative contribution of temperature on dye wastewater decolourization and COD reduction.

To investigate the interactive effect of two factors on the decolourization and degradation of textile dye wastewater, response surface methodology was used and 3D plots were drawn. Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interactive effects of two factors. The response surface curves for the decolourization and degradation of textile dye wastewater were shown in Figs. 1-8. The nature of the response surface curves shows the interaction between the variables.

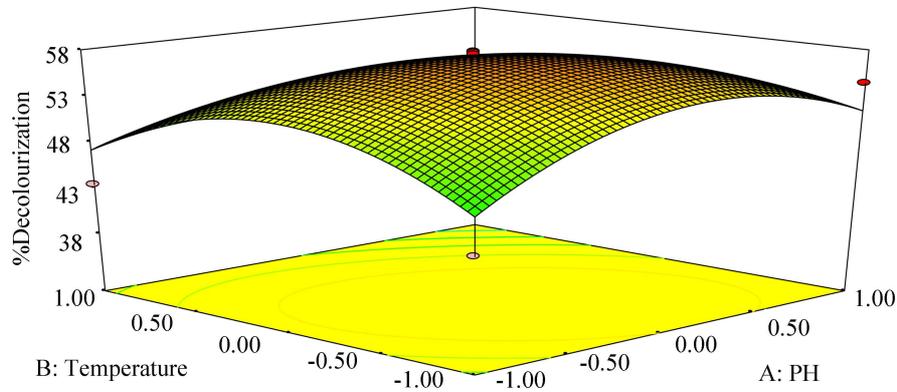


Fig. 1 3D plot showing the interactive effect of pH and temperature on decolourization of textile dye wastewater

The elliptical shape of the curve indicates good interaction between the two variables and circular shape indicates no interaction between the variables. From the figures it is observed that the elliptical nature of the contour in graphs depicts the mutual interactions of all the variables. There is a relative significant interaction between every two variables, and there is a maximum predicted yield as indicated by the surface confined in the smallest ellipse in the contour diagrams.

Fig. 1 shows the interactive effect of pH and temperature on textile dye decolourization. pH is one of the important factor in the treatment of textile dye wastewater by microorganism. pH is very important for fungal growth. They can grow at low pH, normally ranging from 4 to 7 (Zhang *et al.* 1999). From the figure, it was inferred that increase in pH (up to 6.8) increases the dye decolourization efficiency. After that the decolourization efficiency decreases. Similar trend was observed in Figs. 2 and 3. The pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 6 and 7 for most of the dyes. The pH tolerance of decolourizing fungi is quite important for decolourization of textile dye wastewater.

From Fig. 1, it is also observed that the decolourization increases with temperature up to 27.9°C

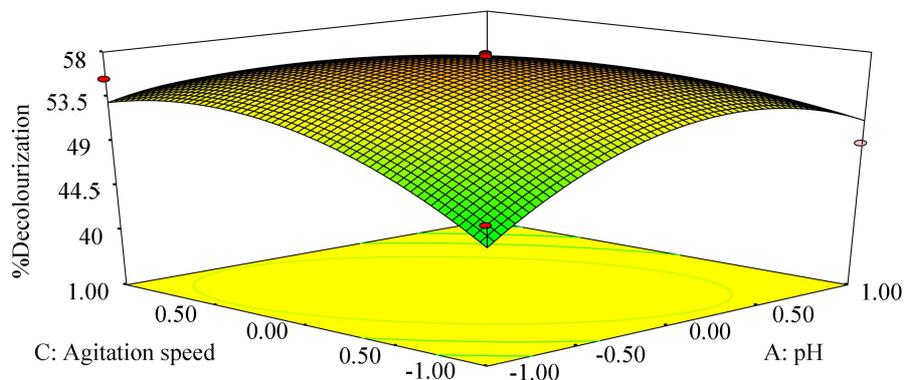


Fig. 2 3D plot showing the interactive effect of pH and agitation speed on decolourization of textile dye wastewater

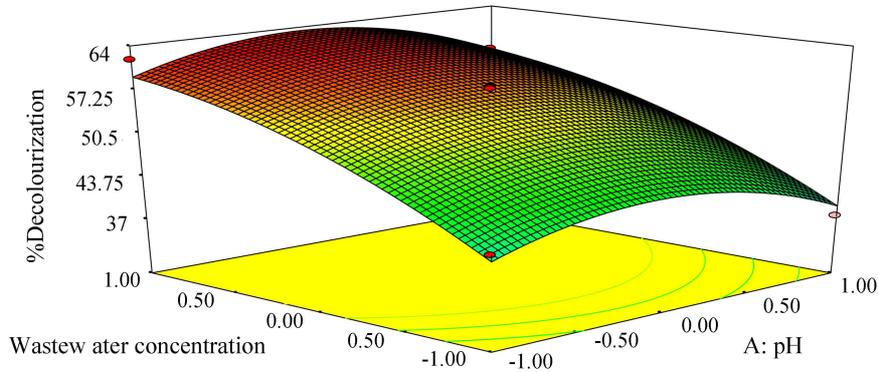


Fig. 3 3D plot showing the interactive effect of pH and wastewater concentration on decolourization of textile dye wastewater

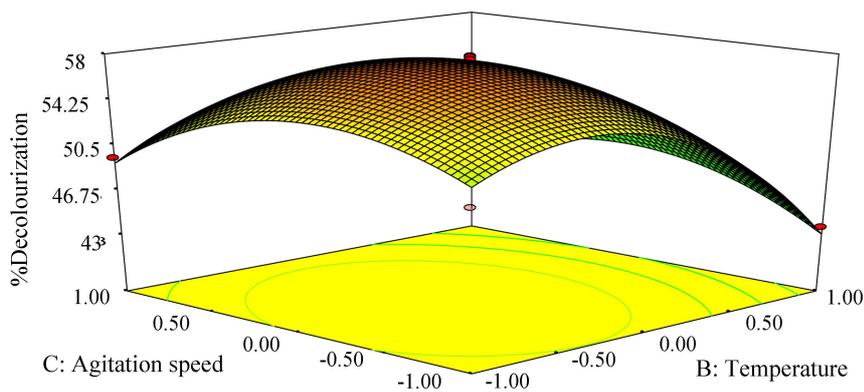


Fig. 4 3D plot showing the interactive effect of temperature and agitation speed on decolourization of textile dye wastewater

and thereafter decreases. Decolourizing activity was significantly suppressed at higher temperatures. This may be due to the loss of cell viability or deactivation of the enzymes responsible for decolourization. Hence the optimum temperature for maximum decolourization of textile dye wastewater using *coriolus versicolor* was 27.9°C. From Fig. 2, it was observed that, the percentage of decolourization increases with increase agitation speed up to 160 rpm. After that point, the dye removal efficiency decreases. This was also observed in Fig. 4. The decrease in dye concentration increases the decolourization. This is clearly depicted in Fig. 3. From the figure it is inferred that the percentage removal of dyes increases with decrease in dye concentration. This is due to, at higher concentrations the chemicals and other pollutants present in the dye wastewater inhibits the growth of microorganism.

The interactive effect of pH and temperature on biodegradation of textile wastewater using *coriolus versicolor* was shown in Fig. 5. From the figure it was observed that increase in pH upto 6.8 increases the COD reduction. Further increase in pH leads to decrease in COD reduction. The interactive effects of other parameters were shown in Figs. 6-8. The trend observed for other

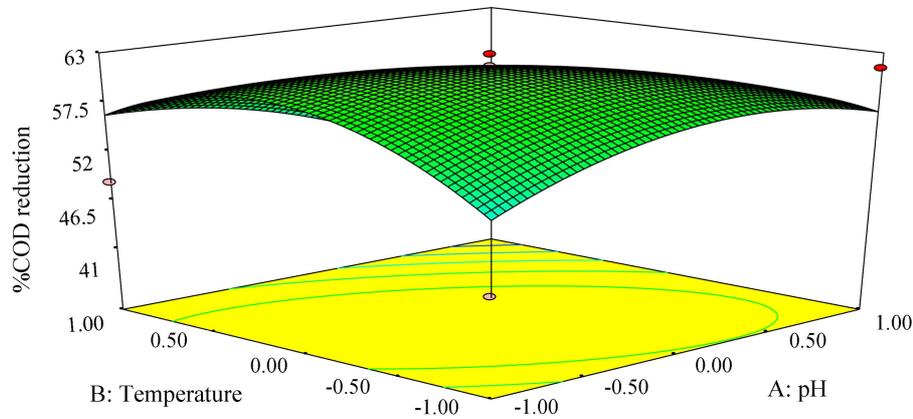


Fig. 5 3D plot showing the interactive effect of pH and temperature on COD reduction of textile dye wastewater

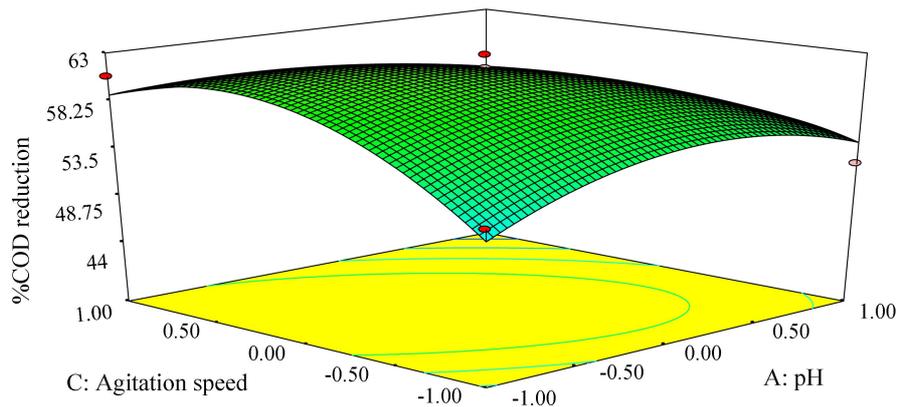


Fig. 6 3D plot showing the interactive effect of pH and agitation speed on COD reduction of textile dye wastewater

parameters was similar to the decolourization profile. The optimum conditions were also same for COD reduction.

The second order polynomial equation obtained from RSM was solved in MATLAB 7.0, to find the optimum conditions. The values obtained from the 3D plot and equations are found to be equal. The optimum condition for the maximum decolourization was: pH – 6.8, temperature – 27.9°C, agitation speed – 160 rpm and dye wastewater concentration – 1:2. The optimal conditions predicted using RSM has been validated using experiments. At the optimized condition, the maximum colour removal and COD reduction were found to be 64.4% and 79.8% respectively.

At the optimum condition, decolourization of textile wastewater was studied by analyzing the supernatants at different time intervals, in an UV - Spectrophotometer in the range of 300 to 800 nm. The results obtained were shown in Fig. 9. A peak is observed at λ_{\max} 395 nm in the UV-Vis spectra. The peak decreases as the day progresses, which showed the decolorization of textile

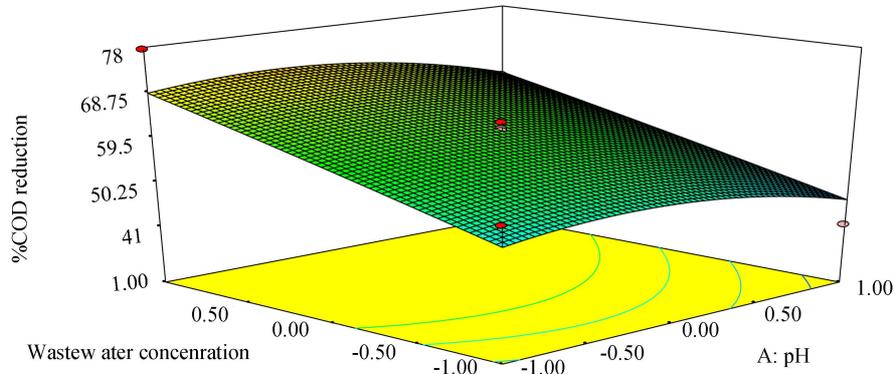


Fig. 7 3D plot showing the interactive effect of pH and wastewater concentration on COD reduction of textile dye wastewater

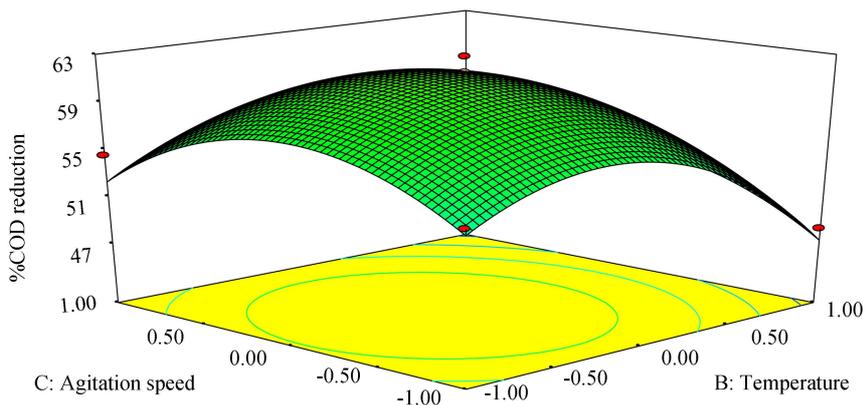


Fig. 8 3D plot showing the interactive effect of temperature and agitation speed on COD reduction of textile dye wastewater

dye using *coriolaris versicolor* at the end of 5th day of operation.

The effect of carbon source on the treatment of wastewater was investigated by the addition of sucrose, glucose, bagasse, pressmud, rice straw and wheat straw. The results obtained in the batch study were shown in Fig. 10. From the figure it was observed that the addition of carbon source increases the percentage decolourization by 5 to 25%. In real textile dye wastewater, the availability of carbon source for the growth of microorganism is found to be low. It needs more carbon for their growth. Hence the addition of external carbon source increases the percentage decolourization of textile dye wastewater. A maximum of 91% decolourization was achieved by the addition of glucose. Similar results were reported by Zhang *et al.* (1999).

4. Kinetics

In this study, first order model and diffusional model were tried to fit the experimental data obtained from the batch degradation of textile dye wastewater using *coriolus versicolor*.

4.1 First order model

The first order model is

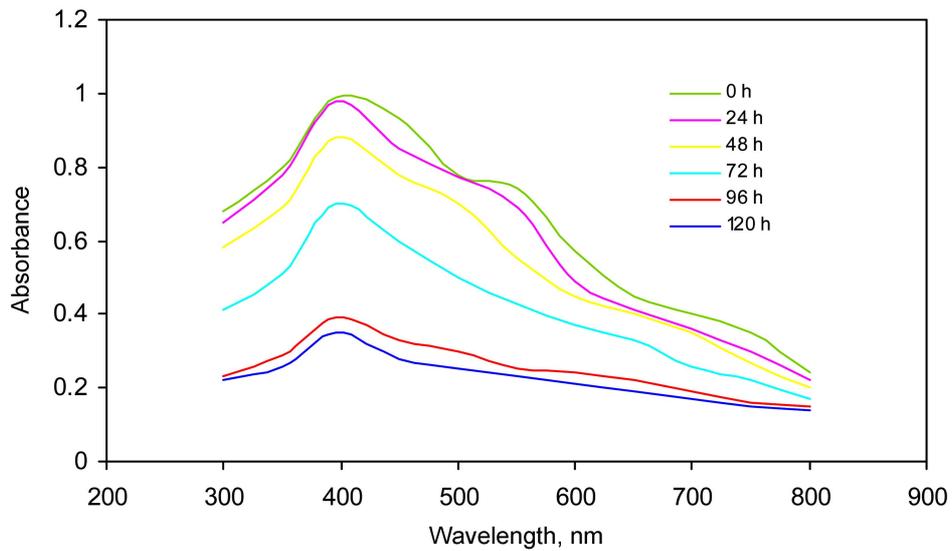


Fig. 9 UV spectra for the decolourization of textile dye wastewater using *coriolus versicolor*

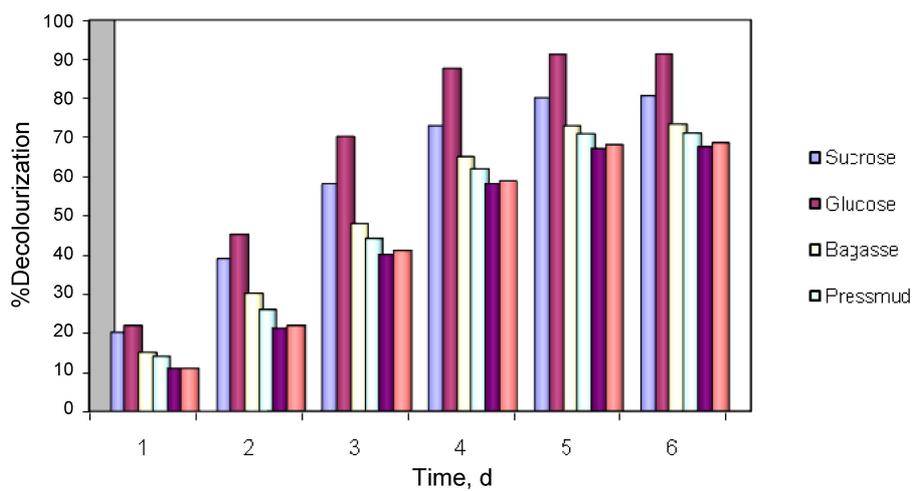


Fig. 10 Effect of carbon source on decolourization of textile dye wastewater using *coriolus versicolor*

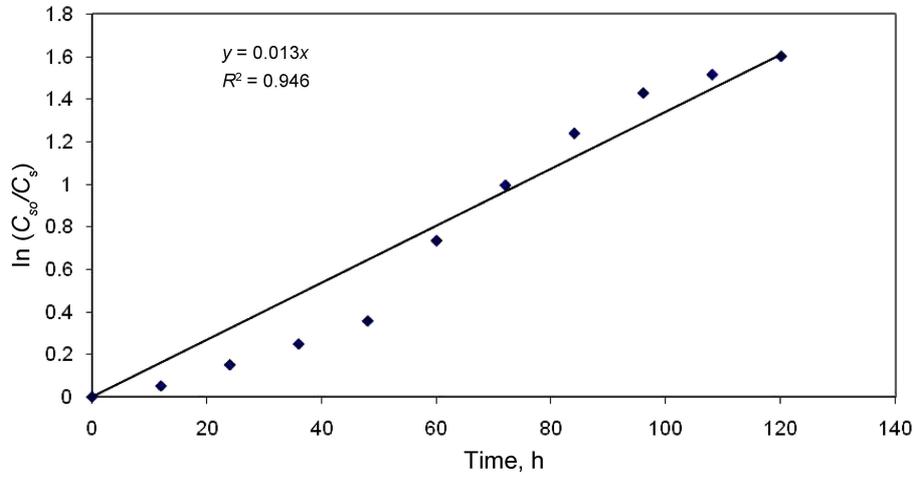


Fig. 11 First order model for the degradation of textile dye wastewater using *coriolus versicolor*

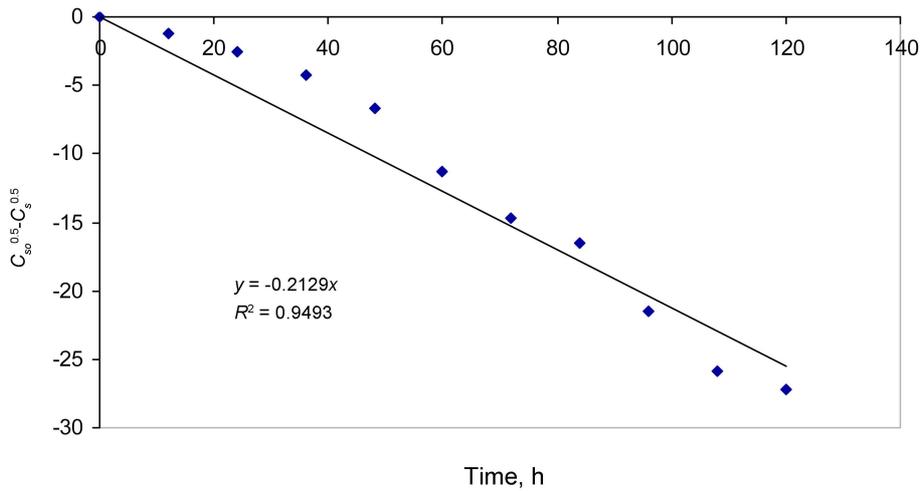


Fig. 12 Diffusional model for the degradation of textile dye wastewater using *coriolus versicolor*

$$-\frac{dC_s}{dt} = k_1 C_s \quad (7)$$

On integration between known limits and rearranging, the above model becomes

$$\ln\left(\frac{C_{so}}{C_s}\right) = k_1 t \quad (8)$$

Where

C_{so} - Initial substrate concentration, mg COD/L

C_s - Substrate concentration, mg COD/L

t - Degradation time, h

k_1 - First order rate constant, h^{-1}

4.2 Diffusional model

The diffusional model is given by

$$-\frac{dC_s}{dt} = k_2 C_s^{0.5} \quad (9)$$

When integrated between the known limits, the above equation becomes

$$\sqrt{C_{so}} - \sqrt{C_s} = \frac{k_2}{2} t \quad (10)$$

Where k_2 = Rate constant for diffusional model

The data obtained from the batch study were fitted to first order model and diffusional model and it was shown in Figs. 11 and 12 respectively. The rate constants, k_1 and k_2 were calculated from the slope of the straight line by the least square (LSQ) fit in the figures. The detailed results including the determination coefficient (R^2) were presented in Table 5. The high R^2 value for the first order indicates the fitness of the model for the degradation process. The negative value of the rate constant for diffusional model shows the inability of the model in describing this process. Hence the degradation of textile dye wastewater follows first order system.

5. Conclusions

In this report, the ability of the white rot fungal, *coriolus versicolor*, was investigated for the treatment of textile dye wastewater. The process parameters pH, temperature, agitation speed and dye wastewater concentration were optimized by RSM. At the optimized condition, a maximum of 64.4% colour removal and 79.8% COD reduction occurs. The UV spectrum confirms the decolourization of textile dye wastewater by *coriolus versicolor*. Kinetics of the textile dye wastewater degradation process was studied by various models. From the results it was found that the degradation follows first order. The results show that *coriolus versicolor* can be effectively utilized for the treatment of textile dye wastewater by the addition of external carbon sources.

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