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Response surface methodological approach for the decolorization of simulated dye effluent using *Aspergillus fumigatus fresenius*

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ABSTRACT

The aim of our research was to study, effect of temperature, pH and initial dye concentration on decolorization of diazo dye Acid Red 151 (AR 151) from simulated dye solution using a fungal isolate *Aspergillus fumigatus fresenius* have been investigated. The central composite design matrix and response surface methodology (RSM) have been applied to design the experiments to evaluate the interactive effects of three most important operating variables: temperature (25–35 °C), pH (4.0–7.0), and initial dye concentration (100–200 mg/L) on the biodegradation of AR 151. The total 20 experiments were conducted in the present study towards the construction of a quadratic model. Very high regression coefficient between the variables and the response (R^2 = 0.9934) indicated excellent evaluation of experimental data by second-order polynomial regression model. The RSM indicated that initial dye concentration of 150 mg/L, pH 5.5 and a temperature of 30 °C were optimal for maximum % decolorization of AR 151 in simulated dye solution, and 84.8% decolorization of AR 151 was observed at optimum growth conditions.

1. Introduction

Synthetic dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetics, and other industries [1]. Worldwide over 10,000 different dyes and pigments are used in textile and printing industries. The total world colorant production is estimated to be 800,000 tons per year and at least 10% of the used dyestuff enters the environment through wastes [2,3]. Most of these dyes are toxic and potentially carcinogenic and their removal from industrial effluents is a major environmental problem [4–6]. Being highly colored, dyes are readily apparent in wastewaters, which is the reason for their breakdown before discharge into the environment. The new environment regulations concerning textile products have banned the discharge of colored waste in natural water bodies [7]. The decolorization of azo dyes by microorganisms also starts by reductive cleavage of azo bond under anaerobic conditions [8,9]. Although, this step leads to decolorization of dye but it generates amines of the dye related structures that are not degraded under anaerobic conditions [10] and tend to accumulate to toxic levels [11]. However, such amines are reported to be readily biotransformed under aerobic conditions [12,13]. To date, most of the research concerning bioremediation with these fungi has centered on a single species *Phanerochaete chrysosporium*, which is known to metabolize a wide range of xenobiotic compounds [14]. There are some other reports related to degradation of textile dye Reactive blue-25 by fungus *Aspergillus ochraceus*. This strain can tolerate very high dye concentrations in distilled water [15]. One of the most important factors that affect fungal decolorization is that dye molecules have different chemical structures. Decolorization of AR 151 was also observed under shaking conditions by the fungal strain *Aspergillus niger* SA1 [29]. Further research is needed to establish the relationships between dye molecular structure and fungal decolorization, and more studies are needed to develop a practical application. At present there is no satisfactory method to economically and reliably decolorize and detoxify textile wastewaters. Synthetic dyes, classified by their chromophores, have different and stable chemical structure to meet various coloring requirements and often are not degraded or removed by conventional physical and chemical processes [16,17].

In this study we have investigated the decolorization of textile diazo dye AR 151 because of its potential to cause toxicity. Recent research point towards the potential of fungal wastewater treatment of textile industries. In biological removal of color from effluents the use of fungi or their oxidative enzymes constitutes an alternative mode of treatment in aerobic conditions. The decolorization can be achieved by two mechanisms, either by adsorption of the dye to the fungal mycelium or by oxidative degradation of the dye molecule [18]. The main objective of the present study was to investigate potential of fungal strain *Aspergillus fumigatus fresenius*

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to decolorize the most important textile diazo dye AR 151 in liquid system under aerobic conditions. We have used indigenous strain *A. fumigatus fresenius* isolated from the soil of dye-contaminated site, so there are some inherent quality in the fungal sp. to decolorize textile dye like AR 151. There are some vital factors that may significantly influence the degradation process, such as temperature, pH and initial dye concentration. In the present investigation maximum dye decolorization ability of the fungus was studied adopting a full range of response surface methodology (RSM) using central composite design (CCD) model to analyze the affectivity of the system under different conditions. The regression model provides an excellent explanation of the relationship between the independent variables and the response [19].

1.1. RSM

Process optimization could be done by empirical or statistical methods. The empirical method is time consuming and does not necessarily enable an effective optimization. A statistics-based procedure called the RSM is a powerful experimental design tool to recognize the performance of composite systems [20–22]. The RSM represents an assemblage of experimental design and multiple regression-based methods that can be applied to evaluate tribulations where several factors might influence a response [23]. In the present investigation, the mutual effect of temperature, pH, and initial dye dosage on color removal from simulated solution by fungal strain *A. fumigatus fresenius* have been studied using CCD in RSM. RSM makes it possible to represent independent process parameters in quantitative form as

$$Y = f(X_1, X_2, X_3, \ldots, X_n) \pm \varepsilon \quad (1)$$

where Y is the response (yield), f is the response function, ε is the experimental error, and $X_1, X_2, X_3, \ldots, X_n$, are independent parameters.

By plotting the predictable response of Y, a surface, known as the response surface is obtained. The form of f is unidentified and may be very complicated. Thus, RSM aim at approximating f by a suitable lower-ordered polynomial in some region of the self-governing process variables. If the response can be well modeled by a linear function of the independent variables, the function can be written as

$$Y = C_0 + C_1X_1 + C_2X_2 + \cdots + C_nX_n \pm \varepsilon \quad (2)$$

A second-order polynomial model where interaction terms have been fitted to the experimental data obtained from the CCD model experiment can be stated in the form of the following equation:

$$Y = C_0 + \sum_{i=1}^{n} C_iX_n + \sum_{i=1}^{n} d_iX_i^2 \pm \varepsilon \quad (3)$$

RSM is a sequential procedure with an initial objective to lead the experiments rapidly and efficiently along a path of improvement towards the general vicinity of the optimum. Optimization of the process can be done by carefully studying the response surface model through different combination of factors for the best response.

2. Materials and methods

2.1. Chemicals

The textile dye Acid Red 151 was supplied by Nahar Fabrics, Lalru (Punjab) India. Other chemicals used were purchased from Loba-Chemi, Bombay, and were of the highest purity available.

2.2. Microorganism

The fungal strain was isolated from dye-contaminated soil collected from within the premises of a textile industry and it was designated as *A. fumigatus fresenius* (MTCC 8190) by the IMTECH, Chandigarh, India. *A. fumigatus fresenius* colonies are spreading broadly, blue green, columnar conodial, conodiospores with echinulate, globule to subglobule conodia. Biochemical tests showed that catalase, oxidase, gelatin hydrolysis and starch hydrolysis tests were positive.

2.3. Culture media and culture conditions

The fungal strain was maintained on potato dextrose agar plates through fortnightly sub-culturing. Further the strain was maintained on PDA slants at 4 °C prior to use. The culture used for inoculation into liquid system was incubated on PDA plates for a week under static conditions at 28 °C prior to inoculation into liquid system. For liquid cultures PDB was used [24].

2.4. Experimental procedure

A stock solution of dye was prepared (1000 mg/L) and desired concentrations of the dye were obtained by further dilutions. For liquid cultures PDB media (2.4%) was used and for dye degradation studies 7 days old cultures were used. Erlenmeyer flasks having 100 ml preautoclaved simulated dye solution of AR 151 in varying concentrations (100–200 mg/L) were inoculated with 10 mm fully sporulated agar plugs (approximately 1.5×10^4 spores) taken from PDA plates. Experiments were performed by varying the temperature (25–35 °C) and pH (4–7) keeping other conditions constant to examine the effect of temperature and pH on dye degradation. pH of the aqueous dye solution was adjusted using 0.1 M HCl and 0.1 M NaOH.

Batch studies were performed to determine the equilibrium time required for maximum dye decolorization. Erlenmeyer flasks containing 100 ml dye solution inoculated with fungal strain *A. fumigatus fresenius* were shaken in an incubator shaker at 160 rpm for the maximum pre-determined time period of 5 days. Samples were withdrawn at fixed time intervals from the flasks, centrifuged and supernatant was analyzed spectrophotometrically for residual dye concentration in the aqueous solution using a Systronics Spectrophotometer-106 at 512 nm (λ_{max} for AR 151). Adsorption of dye to fungal mycelia after incubation for 5 days was not found on all conditions. All the experiments were performed in triplicates and their mean values are reported here. The maximum deviation was found to be ±2.5%. Controls were kept without fungal culture.

Concentration of the dye was calculated from the absorbance, and percentage decolorization (degradation) was calculated by applying the following formulae:

$$\text{Decolorization}\,(\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (4)$$

where C_0 is the initial concentration of dye (mg/L) and C_e is the final concentration of dye (mg/L) at fixed time intervals after degradation.

2.5. Optimization of biodegradation process using RSM approach

In the present study, CCD model for three variables (temperature, pH, and initial dye concentration) was used as experimental design model. In the experimental design model, temperature (25–35 °C), pH (4–7) and initial dye concentration (100–200 mg/L) were taken as input variables. The amount of dye removed (Y) was

Table 1
Experimental range and levels of independent variables

Factors	Range and levels (coded)				
	−1.682	−1	0	+1	+1.682
Temperature (°C), *A*	21.59	25	30	35	38.41
pH, *B*	2.98	4	5.5	7	8.02
Dye (mg/L), *C*	65.91	100	150	200	234.09

taken as the response of the design experiments. A total of 20 experiments were performed. Experimental data obtained from the CCD model experiments can be stated in the form of the following equation:

$$Y = \alpha_0 + \sum_{i=1}^{k} \alpha_i x_i + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \alpha_{ij} x_i x_j + \sum_{i=1}^{k} \alpha_{ii} x_i^2 + \varepsilon \quad (5)$$

Three factors were studied and their low and high levels are given in Table 1. Percent decolorization of AR 151 was studied with a standard RSM design called CCD. Twenty experiments were conducted in triplicates according to the scheme mentioned in Table 2. Design Expert software (Stat Ease, 6.0 trial Version) was used for regression and graphical analysis of the data obtained. The objective of the RSM is to estimate interaction and the quadratic effects as well as giving an idea of the local shape of the response surface. The CCD model had the advantage that it permitted the use of relatively few combinations of variables for determining the complex response function. The optimal values of the chosen variables were obtained by solving the regression equation and by analyzing the response surface contour plots [25]. The inconsistency in dependent variables was explained by the multiple coefficient of determination, R^2 and the model equation was used to forecast the optimum value and afterward to elucidate the interaction involving the variables within the particular range [26].

3. Results and discussions

The color removal efficiencies obtained from the experiments were influenced by the all investigated factors, i.e., temperature, pH and initial dye concentrations and their effects were either individual or interactive.

3.1. Response surface methodological approach for optimization of process variables

3.1.1. Fitting of the quadratic model

In using the RSM approach, the batch runs were conducted in CCD model designed experiments to visualize the effects of independent factors on the response and the results along with the experimental conditions. The experimental results were evaluated and approximating function of dye degradation percent was obtained in the form of following equation:

$$\begin{aligned}\text{Decolorization}\,(\%) = {} & 83.0 - 0.47 \times A + 0.88 \times B - 6.60 \times C - 9.01 \\ & \times A^2 - 14.69 \times B^2 - 4.22 \times C^2 - 0.045 \times A \\ & \times B - 0.73 \times A \times C - 1.30 \times B \times C \end{aligned} \quad (6)$$

where *A*, *B* and *C* are three independent variables. Significance of each coefficient present in Eq. (1), determined by the Student's *t*-test and *p*-values. Analysis of variance (ANOVA) results of this model are presented in Table 3, which indicate that it can be used to navigate the design space. The value of R^2 and adjusted R^2 is close to 1.0, which is very high and has advocated a high correlation between the observed values and the predicted values. This means that regression model provides an excellent explanation of the relationship between the independent variables (factors) and the response (% decolorization).

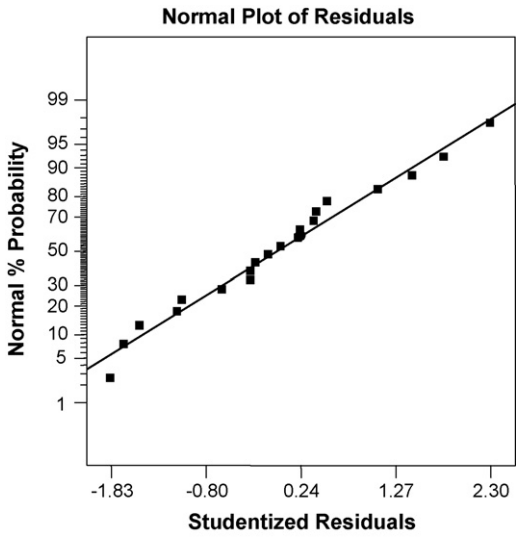

Fig. 1. The studentized residual and normal % probability plot of decolorization of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius*.

Residuals show how well the model satisfies the assumptions of the analysis of variance. Eq. (5) has been used to visualize the effects of experimental factors on degradation percentage response. The model adequacy check is an important part of the data analysis procedure, as the approximating model would give poor or misleading results if it were an inadequate fit. This is done by looking at the residual plots, which are examined for the approximating model [27]. The lack-of-fit term is not significant, as it is desired. The not-significant value of lack of fit (>0.05) revealed that the quadratic model is statistically significant for the response and therefore, it will be used for further analysis. The normal probability and studentized residuals plot is shown in Fig. 1 for % dye degradation. The studentized residuals measure the number of standard deviations separating the actual and predicted values.

Fig. 1 shows that neither response transformation was needed nor there was any apparent problem with normality. The actual (Y) and the predicted (Y_1) dye degradation percent is shown in Table 4. Actual values were the measured response data for a particular run, and the predicted values were evaluated from the model. The values of R^2 (coefficient of determination) and adj R^2 were found to be 0.993 and 0.987, respectively. The fair correlation coefficients might have resulted by the insignificant terms in Table 4, and most likely due to three different variables selected in wide ranges with a limited number of experiments as well as the nonlinear influence of the investigated parameters on process response. It is observed that there are tendencies in the linear regression fit, and the model explains the experimental range studied adequately. The fitted regression equation showed a good fit of the model.

3.1.2. Effect of interactive variables

As discussed in previous section, an experimental design model (CCD) and RSM were used with three process variables to evaluate their effect on the dye degradation process. The response Eq. (6) was obtained for the percentage decolorization. To investigate the interactive effect of two factors on the percentage decolorization, the RSM was used and three dimensional and contour plots were drawn. The inferences so obtained are discussed below.

3.1.2.1. Interactive effect of pH and temperature. To investigate the combined effect of pH of the system and temperature, the RSM was

Table 2
The central composite design matrix for three coded independent variables along with observed response

Experimental run	Coded values of variables			Observed response (*Y*) (%)
	Temperature (*A*)	pH (*B*)	Initial dye concentration (*C*)	
1	−1	−1	−1	57.54
2	1	−1	−1	58.32
3	−1	1	−1	61.84
4	1	1	−1	63.86
5	−1	−1	1	49.04
6	1	−1	1	48.32
7	−1	1	1	49.55
8	1	1	1	47.23
9	−1.682	0	0	60.33
10	+1.682	0	0	56.64
11	0	−1.682	0	41.62
12	0	+1.682	0	43.22
13	0	0	−1.682	84.74
14	0	0	+1.682	59.32
15	0	0	0	82.54
16	0	0	0	83.69
17	0	0	0	84.77
18	0	0	0	82.54
19	0	0	0	83.07
20	0	0	0	81.26

Table 3
One-way ANOVA for RSM parameters fitted to polynomial equation

Sources of variation	Sum of squares	Degree of freedom	Mean square	*F* value	Probability > *F*[*]	
Model	4628.01	9	514.22	166.75	<0.0001	Significant
Lack of fit	23.78	5	4.76	3.37	0.1044	NS[a]
Pure error	7.06	5	1.41			
Residual	30.84		3.08			
Total	4658.85	19				

R^2 = 0.9934; Adj R^2 = 0.9874 and coefficient of variance = 2.75.

[a] Not significant.

[*] Values of "Probability > *F*" less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant.

used and results were shown in the form of contours and 3D plots. Figs. 2 and 3 show that as there was increase in pH, the dye decolorization rate increased with temperature upto optimum level. As temperature increased from 25 upto 30 °C an increase in percent decolorization of AR 151 was observed, after that there was a decrease in the % decolorization rate as the temperature was further increased. The increase in percent degradation was also observed for pH from 4 to 5.5 and decrease in percent decolorization was observed as pH increased beyond 5.5. For instance from Fig. 2 (at pH 4.0, temperature 25 °C) the removal efficiency was 57.5% which increases to 84.8% at pH 5.5 and temperature 30 °C. The optimum value of both the factors, viz., pH and temperature can be analyzed by saddle point or by checking the maxima formed by the *X* and *Y* coordinates.

Table 4
The central composite design matrix for three independent variables along with observed and predicted response

Experimental run	Actual values of variables			Response	
	Temperature (*A*)	pH (*B*)	Initial dye concentration (*C*)	Observed (*Y*) (%)	Predicted (Y_1) (%)
1	25	4	100	57.54	59.24
2	35	4	100	58.32	59.85
3	25	7	100	61.84	63.69
4	35	7	100	63.86	64.11
5	25	4	200	49.04	50.12
6	35	4	200	48.32	47.79
7	25	7	200	49.55	49.34
8	35	7	200	47.23	46.84
9	21.59	5.5	150	60.33	58.35
10	38.41	5.5	150	56.64	56.76
11	30	2.98	150	41.62	40.02
12	30	8.02	150	43.22	42.96
13	30	5.5	65.91	84.74	82.21
14	30	5.5	234.09	59.32	59.99
15	30	5.5	150	82.54	83.03
16	30	5.5	150	83.69	83.03
17	30	5.5	150	84.77	83.03
18	30	5.5	150	82.54	83.03
19	30	5.5	150	83.07	83.03
20	30	5.5	150	81.26	83.03

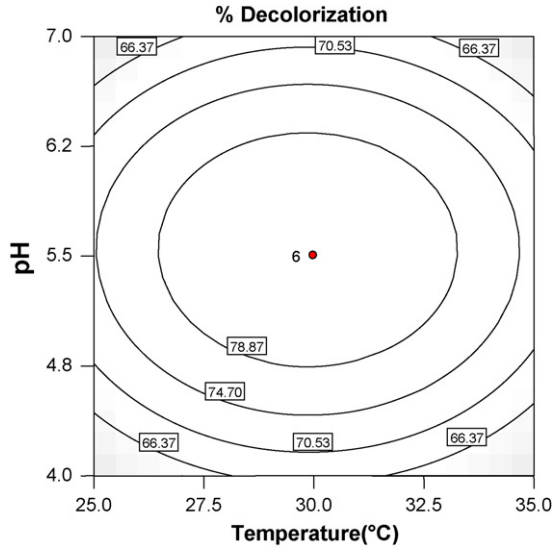

Fig. 2. Contour surface plot for removal of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius* a function of temperature and pH.

3.1.2.2. Interactive effect of temperature and initial dye concentration. Figs. 4 and 5 show the effect of initial dye concentration on percentage decolorization under the predefined conditions given by Design Expert. Graphs show that the maximum % decolorization occurs at the initial dye concentration of 150 mg/L and at temperature of 30 °C, which is in accordance with the model. The decolorization rate decreases with increase in the initial dye concentrations, that might be due to inhibitory effects caused due to toxicity of dye [28]. Temperature of 30 °C and initial dye concentration of 150 mg/L were found to be optimum for maximum % decolorization (84.8%) of AR 151 and this is clearly observable from the saddle point of the figures.

3.1.2.3. Interactive effect of initial dye concentration and pH. Mutual effect of pH and initial dye concentration has been analysed as shown in Figs. 6 and 7 from CCD and it has been estimated that the point of maximum % decolorization of AR 151 was achieved by using the combination of two important process variables, i.e., pH 5.5 and dye concentration of 150 mg/L, respectively that can be analyzed by saddle point or by checking the maxima formed by the *X* and *Y* coordinates.

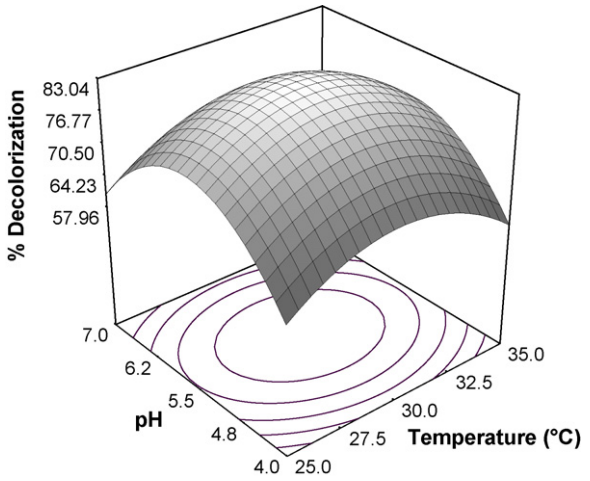

Fig. 3. 3D surface plot for the removal of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius* as a function of temperature and pH.

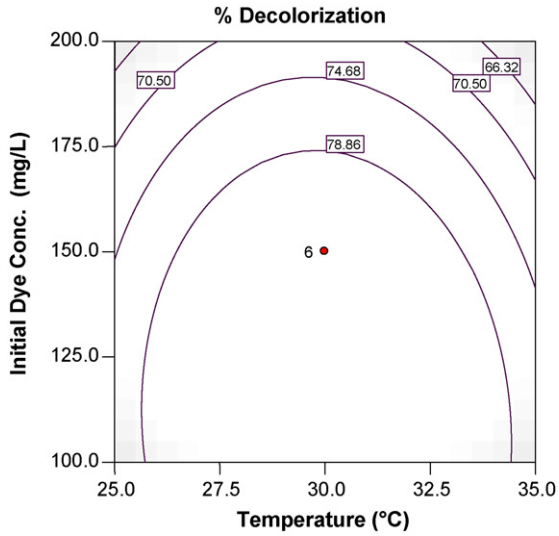

Fig. 4. Contour surface plot for removal of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius* a function of temperature and initial dye concentration.

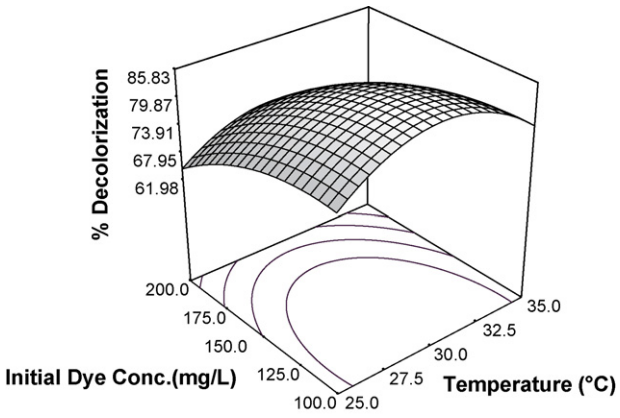

Fig. 5. 3D surface plot for the removal of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius* as a function of temperature and initial dye concentration.

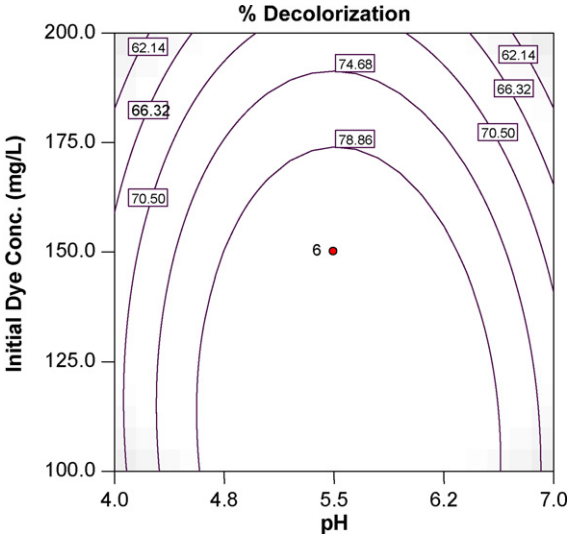

Fig. 6. Contour surface plot for removal of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius* a function of pH and initial dye concentration.

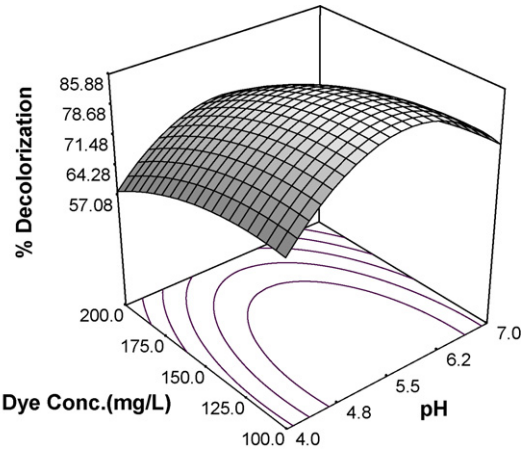

Fig. 7. 3D surface plot for the removal of diazo dye Acid Red 151 by *Aspergillus fumigatus fresenius* as a function of pH and initial dye concentration.

4. Conclusions

The use of an experimental design permitted the rapid screening of a large experimental domain for optimization of the % decolorization ability of fungal strain *A. fumigatus fresenius*. The fit of the model is checked by the determination coefficient (R^2). In this case, the value of the determination coefficient ($R^2 = 0.9934$) indicates that near about negligible of total variations were not explained by the model. The value of the adjusted determination coefficient (adjusted $R^2 = 0.9874$) was also high, showing a high significance of the model. The optimized conditions for highest decolorization (85%) of AR 151 in simulated dye solution are at initial dye concentration of 150 mg/L, pH 5.5 and a temperature of 30 °C. This shows that this fungus has a enormous potential to degrade the textile dyes and resolve the problem of unnecessary dyes present in the effluents of textile industries. Further pilot scale studies are required with this strain for actual industrial applications, and detailed study is needed to explore the mechanism involved.

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