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Optimization of process parameters for efficient decolorization of Congo red by novel fungus *Curvularia* sp. using Box–Behnken design

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ABSTRACT

Response surface methodology (RSM) was employed to investigate the effects of different process variables on the biodecolorization of synthetic dye solution containing Congo red with *Curvularia* sp. RSM, involving Box–Behnken design matrix for three most important influencing process variables; initial dye concentration (20–100 mg l^{-1}), pH (2–8) and temperature (25–40 °C) were successfully carried out for the study and optimization of decolorization process. According to the mathematical models developed by the proposed system, for each process variables showed the effect of each factor and their interactions on color removal. The optimum concentrations of dye, pH, and temperature were found to be 60 mg l^{-1}, 5, and 32.5 °C, respectively, for complete decolorization of Congo red (100%). Predicted values were validated with experimental data, which indicated suitability of the employed model and the success of RSM. High-performance liquid chromatography and Fourier transform infrared analysis confirmed degradation of Congo red.

Keywords: Color removal; *Curvularia*; Concentration; Congo red; Fungi; RSM

1. Introduction

Nowadays, water pollution is increasing due to hurried industrialization and is the major concern of scientific industries. Textile industry is one of the major industries for causing water pollution. It consumes considerable amount of water for wet processing of fabrics for various processes. It was estimated that over 100,000 commercially available dyes existed over 7×10^5 metric tons of dyestuffs produced annually In fact, of the 450,000 tons of organic dyes annually produced worldwide, of which 11% is lost to effluents based on its degree of fixation during manufacture and application processes [1–4].

Textile industry produces huge amount of untreated dyes from dye processing and is released directly into the water body. These effluents cause considerable water and soil pollution because they perhaps toxic, carcinogenic, and mutagenic to living organisms. Moreover, these dye-containing effluents can cause acute and chronic diseases, for example intestinal cancer and cerebral abnormalities in fetus [3–5].

These dye effluents on passage to drinking water cause damage to human food chain as some of these

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dyes are carcinogenic in nature. It is, therefore, essential to remove these dyes from water bodies or treat them in such a way so as to minimize the damage to the environment and also decolorize the water.

Up to date, many physical and chemical methods are existing for the treatment of dye effluent, namely adsorption using activated carbon [6,7] coagulation [8], membrane filtration [9], nano filtration [10,11]. Chemical processes like electrochemical oxidation [12], Fenton reactions [13], photocatalytic oxidation [14,15], and sonochemical processes [16].

But these processes can be summarized as expensive, power-consuming, not completely mineralized and usually not eco-friendly. Therefore, the search for environment friendly and cost-competitive alternative, efficient remedies for dye degradation has been initiated. In recent years, research attention has been focused on biological methods for the treatment of effluents, some of which are in the way to commercialization [17,18].

The active role of fungi in the treatment of wastewater has been extensively researched especially on white rot fungus *Phanerochaete chrysosporium* [19]. Fungus has proved to be a suitable organism for the treatment of textile effluent and dye removal. Several other wood-rotting fungi capable of decolorizing a wide range of structurally different dyes were also isolated and found to be more effective than *P. chrysosporium* [20–22].

Numerous statistically designed experimental models have been applied to optimize the process variables in biological dye degradation processes. In order to determine the influence of process variables by conventional methods, experiments were carried out varying systematically the particular variable and keeping constant the others. These experiments should be repeated to all the influence variables, resulting in an unreliable number of runs. In addition, these exhaustive experimental procedures are not able to find combined effect of the influencing process variables. To overcome these difficulties, effective optimization techniques can be used in order to optimize the process variable for biological research.

Response surface methodology (RSM) is a very useful tool that reduces the several experimental trials needed to evaluate multiple parameters and their interaction. It is a collection of mathematical and statistical techniques useful for modeling and analysis of problems in which a response is influenced by several variables. These RSM designs could be used to find improved or optimal process settings in an efficient use of the resources. RSM methodology has been applied to model and optimize different treatment processes for Congo red and other dyes including biological [23–26], photocatalytic decolorization [27], and sonochemical process [28].

Many researchers have been investigated on this textile azo dye Congo red. *Curvularia* is a facultative pathogen for crops, which is normally found in crop soil. *Curvularia* is found in tropical and subtropical regions, and few are found in temperate zones. Only very few investigation have been done on dye degradation with *Curvularia* strains. However, to the best of our knowledge, the decolorization potential of isolated strain *Curvularia* sp. and the effect of interaction of various parameters using Box–Behnken design during biodecolorization process have not been investigated. Hence, the objective of this study was to investigate the efficient decolorization of Congo red by *Curvularia* with RSM. There are some important factors that may significantly influence the decolorization process, such as temperature, pH and initial dye concentration have been reported. Fourier transform infrared (FT-IR) and high-performance liquid chromatography (HPLC) analysis confirmed the degradation of Congo red.

2. Materials and methods

2.1. Microorganism

The white rot fungus *Curvularia* sp. was obtained from the Bharathidasan University, Tamilnadu, India, and the stock cultures were maintained by periodic subculture on potato dextrose agar medium at 4°C.

2.2. Inoculum

The fungus *Curvularia* was inoculated on potato dextrose agar and incubated at 37°C until extensive spore growth occurred. The basal medium used to study the fungal biomass and decolorization test consists of: D-glucose, 5.0 g l^{-1}; KH_2PO_4, 2.0 g l^{-1}; NH_4Cl, 0.050 g l^{-1}; $MgSO_4{\cdot}7H_2O$, 0.5 g l^{-1}; $CaCl_2.2H_2O$, 0.1 g l^{-1}; thiamine HCl, 100 mg; trace element solution, 10 ml, and the final pH of the medium was maintained at pH 4.5 [23,29]. Trace element solution consisting of $MnSO_4$, 0.5 g l^{-1}; $FeSO_4{\cdot}7H_2O$, 0.1 g l^{-1}; $ZnSO_4{\cdot}7H_2O$, 0.1 g l^{-1} was prepared separately, and 10 ml was added to the medium.

2.3. Dyes and decolorization studies

The structure and properties of Congo red are shown in Table 1. The chemical structure is the sodium salt of benzidinediazo-bis-1-naphtylamine-4-sulfonic acid (C.I. generic name: direct red 28 and C.I.

number 22120). A stock solution of dye Congo red was prepared (1000 mg l^{-1}), and desired concentrations of the dye were obtained by further dilutions. It was added to Erlenmeyer flasks (250 ml) containing 100 ml of the basal medium, which was inoculated with approximately 1×10^5 cells. (Spore suspension of 2 ml approximately added to the dye). The experiments were carried out in an orbital shaking incubator at 150 rpm for 4 d at 37°C. Control experiments for each test were carried out using uninoculated medium with all ingredients including dye. Every 24 h, 5 ml of the sample has been withdrawn and centrifuged at 6,000 rpm for 15 min. The absorbance of the supernatant was spectrophotometrically determined at 498 nm at different time intervals (on the first day and thereafter every 24 h on subsequent days for a total period of 4 d).

2.4. Factorial design

A 3^3 Box–Behnken full factorial design, including three replicate at the central point, was carried out in order to study the parameters (temperature, pH, and initial dye concentration) that influence the decolorization of Congo red by *Curvularia* sp. Five different concentrations of Congo red (20, 40, 60, 80, and 100 mg l^{-1}), pH (2–8), and temperature (25–40°C) were chosen as process parameters and designated as A, B, and C, respectively, as shown in Table 2. Dye decolorization experiments were carried out according to the run order presented in Table 3. The analysis, evaluation and estimation of each coefficient were determined with Design Expert Software version 8. Experiments were performed to validate the accuracy of the experimental value with model predicted value. Data were analyzed using Design Expert 8 program including ANOVA to find out the interaction between the variables and the response and three-dimensional plots were obtained to determine both the interaction and optimal operational factors.

Table 2
Levels and actual values of the variables tested

Factor	Process variables	Range and levels of coded variables		
		−1 (Min.)	0 (Mean)	+1 (Max.)
A	Initial dye concentration, mg l^{-1}	20	60	100
B	pH	2	5	8
C	Temperature, °C	25	32.5	40

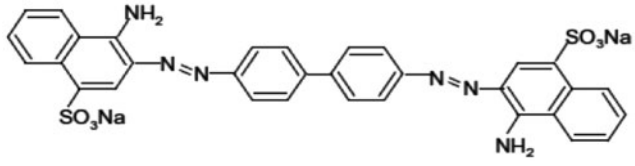
Table 3
Experimental design and results of the Box–Behnken design

Run	Factor A (initial dye concentration)	Factor B (pH)	Factor C (temperature)	Response % removal
1	60	5	32.5	100
2	60	5	32.5	100
3	100	8	32.5	32
4	20	5	40	98.5
5	60	5	32.5	100
6	20	2	32.5	46.9
7	60	5	32.5	100
8	100	2	32.5	45
9	100	5	40	96.8
10	20	5	25	41.45
11	20	8	32.5	55.6
12	60	8	40	36.45
13	100	5	25	31.45
14	60	5	32.5	87.38
15	60	2	40	75.3
16	60	2	25	32.5
17	60	8	25	21

2.5. FTIR and HPLC analysis

The metabolites formed after decolorization of Congo red by *Curvularia* sp. were characterized by using the Fourier transform infrared spectroscopy

Table 1
Structure, properties, and maximum absorbance of Congo red

S. no.	Dye	Molecular structure	Molecular weight	Absorption maxima (λ_{max})
1	Congo red		696.68	498 nm

(FTIR). FTIR analysis was carried out in the mid-IR region of 400–4,000 cm^{-1} with 16 scan speed. The pellets prepared using spectroscopic pure KBr (5:95) were fixed in sample holder, and analyses were carried out. HPLC analysis was carried out (Shimadzu model) on C18 column (symmetry, 4.6 × 250 mm) by using gradient of methanol and acetonitrile (75:25) with flow rate of 1 ml/min for 10 min.

3. Results and discussion

Box–Behnken design was constructed to determine the process variables for color removal. Among the variables screened, the most influencing factors for decolorization with high significance level were in the order of initial dye concentration, pH, and temperature.

3.1. Effect on initial concentrations of the dye

Box–Behnken design has been successfully applied for optimizing conditions for biodecolorization of Congo red with isolated strain *Curvularia* sp. The experimental results were analyzed with RSM to obtain an empirical model for the best response. By applying multiple regression analysis methods, the predicted second-order polynomial response equation for actual factors are:

$$\begin{aligned}\%\ \text{removal} = {} & 489.15865 + 0.91426 \times \text{concentration} \\ & + 54.04153 \times \text{pH} + 23.63641 \\ & \times \text{temperature} - 0.045208 \\ & \times \text{concentration} \times \text{pH} + 5.66667E - 003 \\ & \times \text{concentration} \times \text{temperature} - 0.30389 \\ & \times \text{pH} \times \text{temperature} - 8.27766E - 003 \\ & \times \text{concentration}^2 - 4.37297 \times \text{pH}^2 \\ & - 0.29879 \times \text{temperature}^2\end{aligned} \quad (1)$$

As shown in Eq. (1), the combined effect of temperature and concentration and temperature^2 was negligible. Hence, the equation can be written in terms of influencing parameters.

$$\begin{aligned}\%\ \text{removal} = {} & -489.15865 + 0.91426 \times \text{concentration} \\ & + 54.04153 \times \text{pH} + 23.63641 \\ & \times \text{temperature} - 0.045208 \\ & \times \text{concentration} \times \text{pH} - 0.30389 \times \text{pH} \\ & \times \text{temperature} - 4.37297 \times \text{pH}^2 - 0.29879 \\ & \times \text{temperature}^2\end{aligned} \quad (2)$$

The analysis of variance (ANOVA) of regression model demonstrates that the model is highly significant, as is evident from the Fisher's *F*-test with a very low probability value [$P_{\text{model}} = 0.0009$] with 95% of confidence interval. A *p* value less than 0.01 indicate that the model is statistically significant. According to the ANOVA results, the models present high determination coefficients (R^2): 0.937 for the decolorization of Congo red (shown in Table 4). The combined effect of initial concentration of dye with pH, initial concentration of dye with temperature, and temperature with pH was also analyzed. In traditional optimization techniques, one variable is changed at a time by keeping remaining parameters as constant, whereas RSM examines the simultaneous influence of all the involved variables and hence, the main effect plot obtained in this investigation describes not only the effect of change of one variable but a combined effect of change in both the variables. There was a significant decrease in the % decolorization with an increase in pH [25–29].

Using RSM, the combined effect of three variables can be predicted which is difficult to observe in conventional methods. The effects of these influencing variables on Congo red decolorization are shown through Figs. 1((a) and (b)) and 2((a) and (b)) show the 3-D response surface plot of interactions between varying concentrations of dye with pH and temperature. The surface plot and contour plots shows the decrease in dye decolorization with increase in dye concentration, while the decolorization increased with the increase in temperature from 25 to 32.5˚C. Maximum decolorization was observed at 60 mg l^{-1} dye and pH 5. Decolorization was decreased with the increase in temperature and decrease in pH. At a high dye concentration, the efficiency of enzyme increased and completely diminished at pH 5 and temperature of 40˚C. The surface plot also shows the best decolorization (97.48%) obtained at 60 mg l^{-1} dye concentration, pH 5, and temperature 32.5˚C which is corresponding with the experimentally obtained response (100%) under the conditions used. Previously, Li et al. [30] had reported that increasing Congo red concentration from 25 to 50 mg l^{-1} leads to increase in dye decolorization. Congo red had shown considerable decrease in color by the aerobic liquid cultures of *Curvularia*.

3.2. Effect of pH

The fungal ligninolytic enzymes show maximal activity at low pH. Therefore, efficient dye decolorization is also observed at low pH. The efficiency decreased from 59 to 8% as pH was increased from 5

Table 4
Analysis of variance

Source	Sum of squares	Degrees of freedom	Mean square	*F* Value	*P*-value	Prob > *F*
Model	14184.99	9	1576.11	11.61	0.0019	Significant
A	187.21	1	187.21	1.38	0.0086	
B	373.33	1	4147.33	30.55	0.0009	
C	4147.33	1	4147.33	30.55	0.0009	
Residual	950.14	7	135.73			
Lack of fit	822.73	3	274.24			
Pure error	127.41	4	31.85			
Total	15135.13	16				

R^2: 0.937; adj R^2: 0.9191

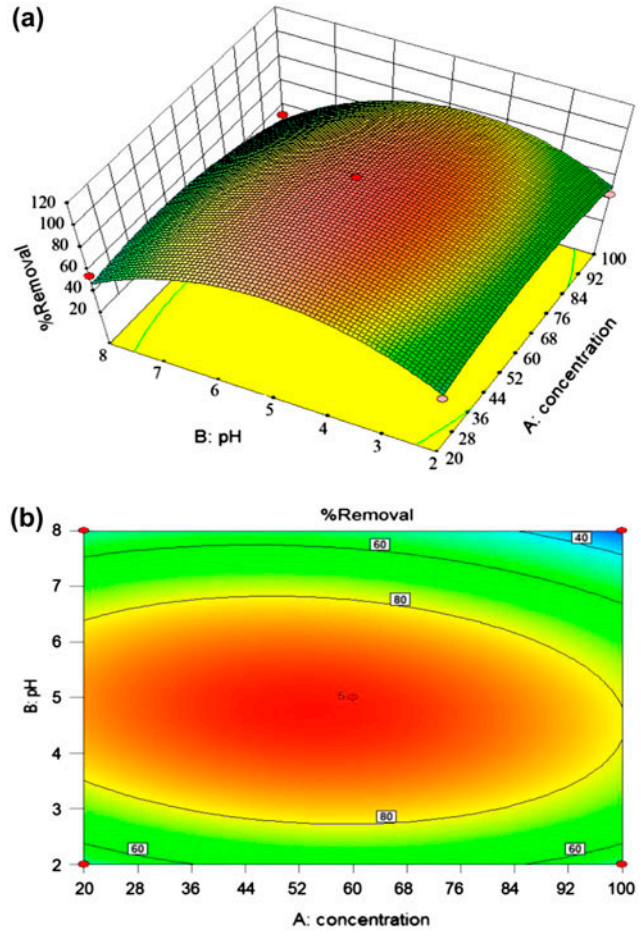


Fig. 1. (a) 3D response surface plot for decolorization of Congo red by *Curvularia* sp. as a function of initial dye concentration and pH at 32.5℃. (b) Response contour plot for initial dye concentration versus pH.

to 6 [30,31,33,34]. Hence, it was observed that for majority of the fungi the optimum pH for dye decolorization lies in the acidic range. Figs. 1((a) and (b)) and 3((a) and (b)) represent the response surface for Congo red decolorization. Surface plots show the decrease in dye decolorization with increasing pH values. Plots 1 (a) and (b) clearly show that the decolorization by *Curvularia* was sensitive even to small alterations of the pH. As it can be seen from plots 1(a) and (b), the temperature slightly influenced the decolorization. On the other hand, the initial dye concentration did not affect the decolorization in the treated range. From the results, it can be observed that pH plays vital role. At basic conditions (pH > 5), a little decolorization was observed for Congo red [27,28].

3.3. Effect of temperature

Effect of temperature on dye decolorization was extensively studied by many researchers. Majority of these reports indicates that the decolorization capacity of the fungal biomass increases with increase in temperature [25,29,32]. In order to study the variation in temperature on decolorization, studies were carried out at temperatures ranging from 25 to 40℃. From the results presented in Figs. 2((a) and (b)) and 3((a) and (b)), it can be concluded that the temperature markedly influences an important role on decolorization of Congo red in the range 25–40℃. At higher (>40℃) or lower (<25℃) temperatures, the decolorization activity of the fungus gets reduced, which indicate that either the fungus is not able to grow for decolorization or they get denatured. Based on the results of the screening study, for examining the effect of temperature, it was varied between 25 and 40℃, and it can be noted that Congo red showed a higher rate of decolorization of 100% at 32.2℃. It has shown very poor decolorization of 21% at 25℃ and 36.45 at 40℃ in two days.

3.4. Model validation

The main objective of the optimization studies is to determine the optimum values of influencing variables

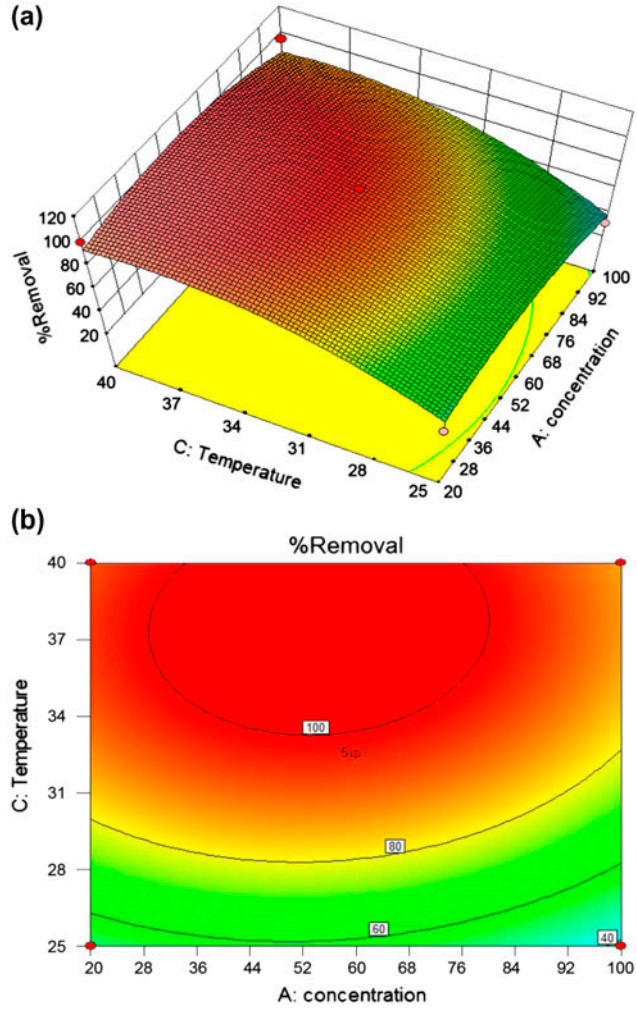


Fig. 2. (a) 3D response surface plot for the decolorization of Congo red by *Curvularia* sp. as a function of initial dye concentration and temperature at pH 5. (b) Response contour plot for initial dye concentration versus temperature.

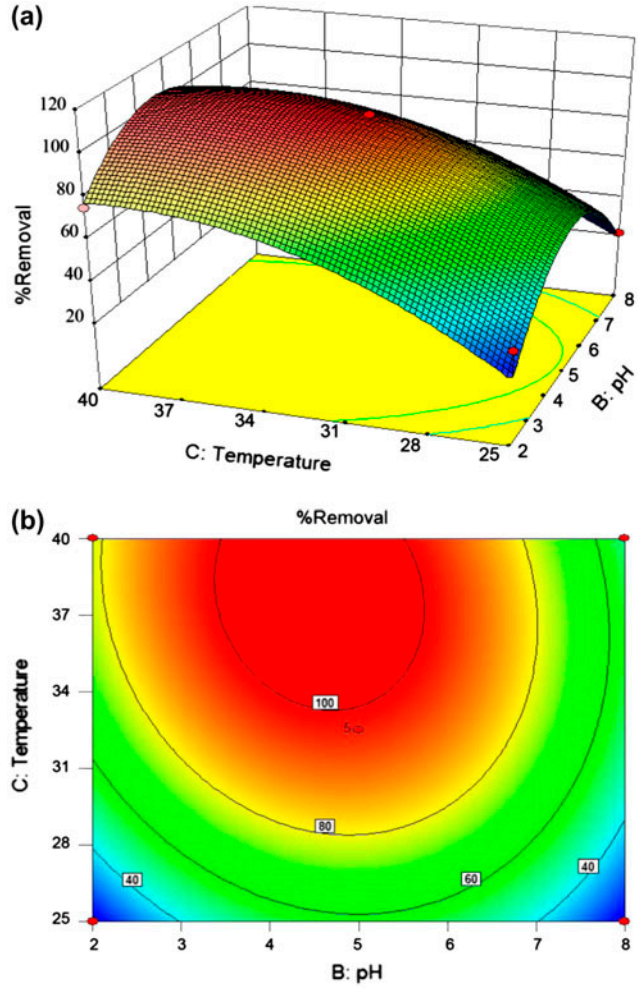


Fig. 3. (a) 3D response surface plot for the decolorization of Congo red by *Curvularia* sp. as a function of temperature and pH at 60 mg l^{-1}. (b) Response contour plot for temperature versus pH.

for biodecolorization of Congo red using *Curvularia* sp. from the model obtained and to maximize the removal. The evaluation of standard error of design is shown in Fig. 4. The results of theoretically predicted responses and experimentally determined are shown in Fig. 5. To validate the optimum combination of the variables, confirmatory experiments must be carried out. For this purpose, new experiments were conducted in triplicate to verify the optimum conditions, indicating the validity and adequacy of the predicted models. According to the models, the optimum conditions for the Congo red dye decolorization were as follows: temperature 32.5°C, initial dye concentration 60 mg l^{-1}, and pH 5, predicting 100% decolorization. For the entire range of the tested process variables, the experimental results are very close to the predicted

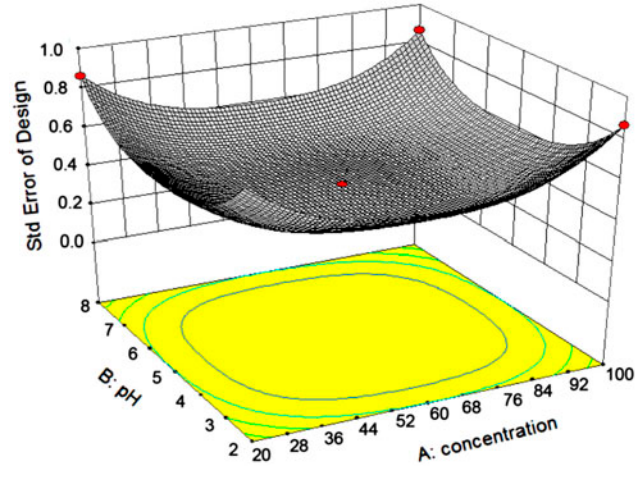


Fig. 4. Plot of standard error of design.

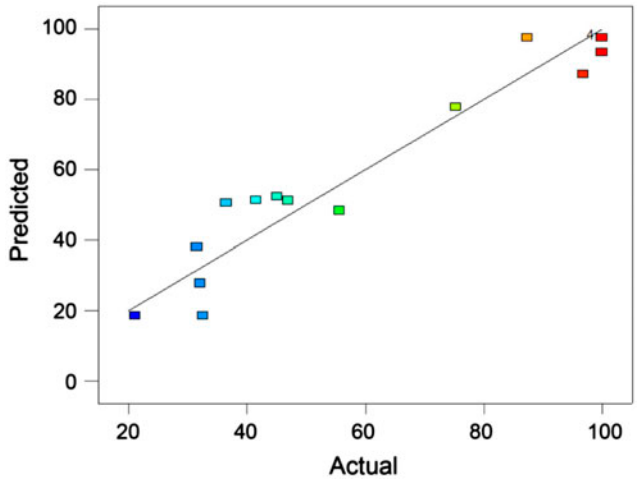


Fig. 5. Plot of actual versus predicted values.

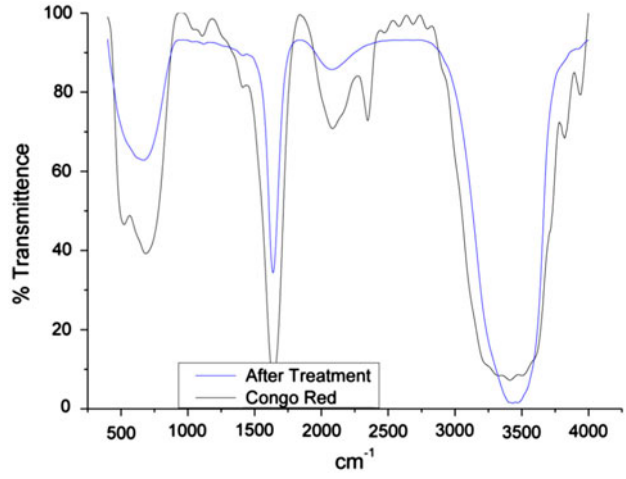


Fig. 7. FTIR analyses of Congo red before (shown in black line) and after treatment (shown in blue line).

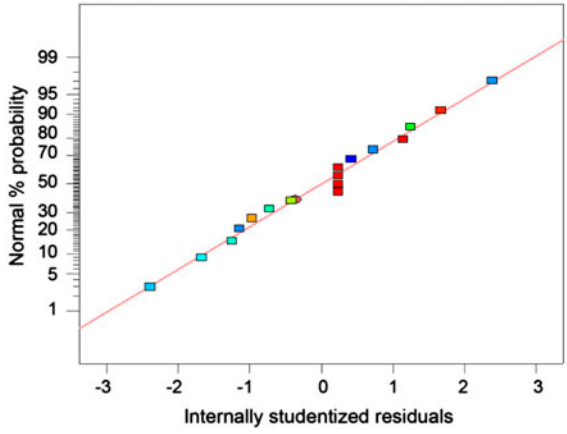


Fig. 6. Plot of internally studentized residuals against model predicted value.

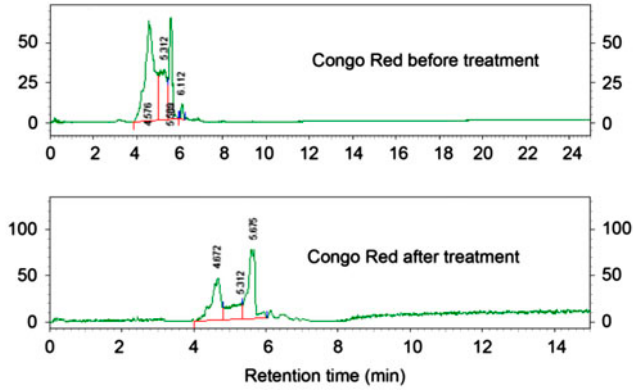


Fig. 8. HPLC analyses of Congo red before treatment (top) and after (bottom) treatment.

values obtained from the models. By constructing a normal probability plot of the residuals, a check was made for the normality assumption as shown in Fig. 6. The normality of the model assumption was in agreement with the internal studentized residual plot validated along with a straight line [23–30].

3.5. FTIR and HPLC analysis

The FTIR spectrum of the control dye compared with extracted metabolites confirmed the degradation of Congo red and is shown in Fig. 7. The difference in FTIR spectrum of Congo red and metabolites obtained after its decolorization resulted in biodegradation. The FTIR spectrum of Congo red showed specific peaks. This was supported by the peaks at 684 cm^{-1} for C=C–H bending alkynes, 522 cm^{-1} for C–Br stretching vibrations for alkyl halides, 1,107 cm^{-1} for S=O stretching vibrations of aliphatic amines, 1,639 cm^{-1} for N–H bending primary amines, 2083 cm^{-1} for C=C stretching alkynes vibrations, 2,579 cm^{-1} for H–C=O:C–H stretching vibrations, 3,411 cm^{-1} for N–H stretching vibrations of primary amine. The FTIR spectrum of metabolites obtained after decolorization of Congo red showed peaks at 668 cm^{-1} for C–H "oop" aromatics, C–Br stretch alkyl halides, 1,637 cm^{-1} N–H stretching primary amines, 3,432 cm^{-1} for O–H stretching vibrations of primary amide [33].

As shown in Fig. 8, the HPLC analysis of dye sample collected at 0 h incubation showed peak at

5.589 min. As the decolorization progressed, the biodegradation of parent compound was observed with different detectable peaks at different retention times (4.672, 5.675) [34].

4. Conclusions

Several investigations were performed on the ability of white-rot fungi to decolorize and degrade textile dyes. However, to the best of our knowledge, the decolorization potential of *Curvularia* sp. and the effect of interaction of various process parameters using Box–Behnken design during biodecolorization process have not been reported. Mycelium of this fungus was able to decolorize the azo dyes. The results indicate that this is a possible and potential application in the bioremediation of textile effluents containing azo dyes and other toxic compounds. The Box–Behnken statistical experimental design and RSM were found to be efficient tools to optimize these experimental conditions. Results revealed that the Box–Behnken design could effectively be applied for the modeling and optimization of biodecolorization process, with the fewest number of experiments and showed a satisfactory agreement of second-order regression model with experimental results. Hundred per cent decolorization can be achieved at the following optimum condition: initial dye concentration 60 mg l^{-1}, temperature 32.5°C, pH 5, and an inoculum size of 2 ml (approximately 1.0×10^5 cell/ml) (pre determined) thus the high effectiveness in decolorization is attained.

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