Application of Response Surface Methodology for Biosorption of Reactive Dyes from Textile Effluent Using Dead Fungal Biomass of *Rhizopus Arrhizus* NCIM 997

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Abstract: **- Response Surface Methodology was employed for studying the biosorption of reactive dyes from textile effluent by utilization of dead biomass of *Rhizopus arrhizus* in a batch system. Central Composite Design at the specified combinations of four variables (pH, biosorbent dosage, speed of agitation, contact time) was adopted to achieve maximum biosorption. The fitted quadratic model (P<0.0001) was used to arrive at the best operating conditions. Under the following optimum conditions i.e., pH 2.0; biosorbent dosage 3 g /L; speed of agitation 80 rpm and contact time 60 min, 99.60% of the dyes were removed from the wastewater. The mechanism of biosorption was elucidated by FTIR, XRD and BET analysis. This work demonstrated the feasibility of employing *Rhizopus arrhizus* as an effective and economical fungal biosorbent for the removal of dyes from the textile effluent.**

Keywords*: Biosorption, Textile effluent, Response surface methodology, *Rhizopus arrhizus,

I. INTRODUCTION

Textile industry extensively uses reactive dyes, owing to their technical attributes and easy availability of raw materials [1].The effluent generated by reactive dyeing processes contains hydrolyzed dyes amounting to 20-30% of the dyes used; recalcitrant organics; surfactants, sizing, coating and finishing agents, which are responsible for the high COD and BOD of the effluents; textile fibres (60-100 g/L); electrolytes, essentially NaCl and Na_2CO_3, which contribute to the high salinity of the wastewater [1-6]. Thus, release of textile effluents in the ecosystem has raised a great concern due to the aesthetic considerations as well as their recalcitrant nature; potential toxicity, carcinogenicity and mutagenicity to animals and humans [7-9]. Reactive dyes because of their complex aromatic molecular structure tend to pass through the conventional physical and chemical processes while inhibiting the conventional biological wastewater treatment processes [7, 10-14]. This has given an impetus to use eco-friendly techniques such as adsorption because of ease of design, effectiveness, simplicity of operation, insensitivity to noxious materials and capacity to treat the dyes in a more concentrated form [15]. One of the most extensively used adsorbents is activated carbon because of its very good adsorption capacity for organic pollutants. However, its commercial application is limited due to high expenses, difficulty in regeneration and problems with respect to its ultimate disposal [16-18]. Hence, low-cost biosorbent materials such as natural waste materials originated from agriculture and industry (i.e. corncob, pinewood, rice husk, bagasse, chitosan, etc.) as well as biosorbents produced from microbial biomass have gained increase in attention due to reduction the adsorbent dose and minimization of the disposal problem [4,19-21]. Among the various types of biomasses, the inactivated fungal biomass is particularly attractive due to its constant and cheap supply from industrial fermentation processes, selectivity, high removal rates, ease of storage and regeneration. The effectiveness of the biosorption by some fungi under equilibrium conditions has been shown to be superior to the traditional ion-exchange resins and activated carbon for reactive azo dyes [4,19, 22, 23].

The majority of wastewater treatment processes are multi-variable and optimization of the process by statistical approaches such as Response Surface Methodology (RSM), offers advantages such as closer confirmation of the output response to normal, lower process variability, reduced development time and costs. RSM helps to optimize operational variables, to build models, to scrutinize the interactions between the variables while minimizing the empiricism of trial-and-error techniques [24,25]. Recently, several wastewater treatment processes including; textile, tannery, palm oil mill and industrial paint effluents, landfill leachate, etc. have been optimized via RSM [26,27]. The current work focuses on the application of RSM in studying the usability and effectiveness of the biosorbent made from the dead biomass of *Rhizopus arrhizus* in optimization of textile wastewater treatment and modeling the process parameters. The kinetics data were used to identify the

adsorption mechanism and the rate limiting step. Surface characterization of the biomass was done using FTIR, XRD and BET analyses.

II. MATERIALS AND METHODS

1. Materials

Rhizopus arrhizus NCIM 997 used as a biosorbent in this study was obtained from National Chemical Laboratory, Pune, India. Textile effluent was directly collected in airtight plastic bottles from the outlet before it was subjected for further treatment from DyStar Pvt. Ltd., Rabale, India. The structure of the dyes in the effluent was not revealed for commercial purposes. All the chemicals used in this study were of analytical grade and procured from HiMedia, India.

2. Preparation of the biosorbent

Fungal biomass was acquired by aseptically transferring mycelia from the potato dextrose agar (PDA) spread-plate cultures to 100 mL of potato dextrose broth (PDB) (g /L: potato infusion from 200 g potatoes, dextrose 20, yeast extracts 0.1, pH 5.0) containing 0.25 % Tween 80 (to prevent sporulation) in 250 mL Erlenmeyer flasks. The biomass was harvested after seven days of incubation at 30±1^{o}C under static conditions with sporadic shaking. Fungal biomass was then washed thoroughly with double distilled water and dried at 80^{o}C for 24 h. The size of the biomass particles was kept uniform by grinding into a fine powder and sieving through a 150-mesh sieve. Dried biomass was then preserved in a desiccator and was used without any physical or chemical treatment for the biosorption experiments [28].

3. Characterization of the biosorbent

The Fourier transform infrared spectroscopy (FTIR) analysis was used to identify different functional groups present on the biosorbent before and after biosorption of the dyes in the textile effluent. The analysis was carried out using the KBr, with the spectral range varying from 4,000 cm^{-1} to 400 cm^{-1}. The infrared spectra were obtained and averaged over 32 scans in the transmission mode. In order to assess the presence of crystalline phases present in the biosorbent before and after biosorption, X-ray diffraction patterns were obtained with a diffractometer (Rigaku Miniflex X-ray diffractometer) with the 2θ angle varying between 5^{o}and 80^{o}.The BET (Brunauer, Emmett and Teller) technique that measures the quantity of gas molecules needed to saturate the solid surface under equilibrium conditions was used to analyze the surface area of the biomass. A known quantity of the sample (0.0001 g), after drying in an oven at 80^{o}C for 24 h, was evacuated and then saturated with liquid nitrogen. The quantity of the gas required for saturation of the sample was taken as a measure of surface area using the BET Surface Area Dual Component Gas Analyzer Model SAA-2002.

4. Characterization of the textile effluent

The effluent used in this study was a multi-component solution containing commercial reactive dyes (used to dye cotton fabric at 60 ^{0}C for 90 min) viz. Remazol G.Y. RGB, Remazol Navy RGB, Remazol Blue RGB, Remazol Red RGB, Glauber salt (80g /L) and soda ash (20g /L).The temperature was measured with laboratory thermometer and pH was determined using pH meter (Hanna digital pH meter, model-HI 9125) at the sampling site. The sample was transported to laboratory and stored at 4±1°C until use in accordance with the standard methods [29]. The effluent was immediately subjected to physico-chemical analysis following standard APHA methods (APHA, 2002) after filtering to remove large suspended particles [30]. The textile effluent was characterized for odor, color, chlorides, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD) (Table 2). The effluent was diluted 1:10 for further experiments. The color of the sample was checked from time to time before every experiment to ascertain sample integrity.

5. Batch biosorption studies

The batch biosorption experiments were carried out at the specified combinations of physical parameters to evaluate the potential of the biosorbent made from the dead biomass of *R. arrhizus* in removing color from the textile effluent. 250 mL Erlenmeyer flasks containing 50 mLof 1:10 diluted effluent at desired pH and concentration of biosorbent dosage were kept under agitation in a rotating orbital shaker (Labtop Quality Lab Equipment, India) for desired period of time for these experiments. The residual concentration of dyes in the effluent was determined after centrifugation at 4000 rpm using modified ADMI (American Dye Manufacturers' Institute) spectroscopic method [31,32]. The ADMI values are a true measurement of water color due to the presence of colored minerals and dyes, humic breakdown substances and iron, independent of hue and thus give a more accurate description of wastewater and colored mixture. The change in hue of the effluent was determined by spectrophotometric comparison of the sample with calibration curve prepared by measuring the absorbance of known concentrations of the APHA/Pt-Co liquid color standards at 450 nm using a UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). Simultaneously blank without biosorbent was run as a control. The unit of color in platinum-cobalt method of measuring color is that produced by 1 mg platinum /L in the form of the chloroplatinate ion was calculated as:

$$Colourunit = \frac{A*50}{B} \quad (1)$$

Where, *A* = Estimated color of diluted sample; B = mL of sample taken for dilution.

The response expressed as percent decolorization was calculated as:

$$ADMI\ removal\ (\%) = \frac{ADMI\,(t_0) - ADMI\,(t)}{Absorbance\ (t_0)} * 100 \qquad (2)$$

Where, Absorbance (t_0) and Absorbance (t) are the initial absorbance value (at 0 h) and the absorbance value after a particular reaction time (t), respectively.

6. Central Composite Design (CCD) and statistical analysis

The optimal levels of the significant variables and their interactions on the biosorption process were investigated using Central Composite Design (CCD) [33]. In order to make the design rotatable, a value of $\alpha = (nF)^{1/4}=2$ was taken. A four factor, five level CCD consisting of 30 runs, with 6 center points (4 cube points and 2 axial points) was conducted to locate true optimum values of pH (X_1), biosorbent dosage (X_2), speed of agitation (X_3) and contact time (X_4) (Table 1). Randomized run order was used to minimize the effects of variability in the observed responses due to extraneous factors. To check for the adequacy of the model and to estimate the experimental error, replicates were used. According to this design, the total number of treatment combinations were $2^k + 2k + n_0$, where k is the number of independent variables and n_0 is the number of repetitions of experiments at the center point [34]. A second-order polynomial response equation (Eq.3) was proposed to correlate the dependent and independent variables:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i 2 + \Sigma\beta_{ij} x_i x_j,\ i = 1, 2, 3. . .k, \qquad (3)$$

Where, Y is the predicted response, β_0 is the intercept, x_i and x_j are the coded independent factors, β_i is the linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the interaction coefficient.

Table 1.Experimental range and levels of independent process variables tested in Central Composite Design

Designation	Variable	-2	-1	0	1	2
X1	pH	1.0	1.5	2.0	2.5	3.0
X2	Biosorbent dosage (g /L)	2.0	3.0	4.0	5.0	6.0
X3	Speed of agitation (rpm)	70	75	80	85	90
X4	Contact time (min)	30	60	90	120	150

The quality of the polynomial equation was judged by determination coefficient (R^2) and its statistical significance was checked by Fischer's F-test. Analysis of variance (ANOVA) was conducted to determine the significance of the model. The response surface plots of the model-predicted responses were utilized to assess the interactive relationships between the significant variables. The results obtained from the statistical analyses of CCD were verified by validation tests, using the predicted optimized conditions against the basal conditions. Minitab 16 (State College, PA, USA) and Design Expert Version 6.0.8 (Stat-Ease Inc., Minneapolis, USA) software were used for experimental design, construction of quadratic models and graphical analysis of the experimental data. Response surface plots of the model-predicted responses were utilized to assess the interactive relationships between the variables [35].

III. RESULTS AND DISCUSSION

1. Characterization of the textile effluent

The initial characterization of the dark bluish purple colored effluent collected from DyStar India Ltd., Mumbai is shown in Table 2. The effluent had objectionable odor probably due to the presence of volatile compounds, which is aesthetically unacceptable. The temperature of the raw textile dyeing effluent was 40^{o}C, indicating potential to pose harm to the aquatic life [36,37]. One of the important factors serving as an index for pollution and a determinant for effluent treatment is pH. A pH of 11.23 associated with the effluent was above the tolerance limits of (5.5-9.0) prescribed by the Bureau of Indian Standards (BIS) (1981) for the discharge of industrial effluents. The waste water used in this study was highly turbid and showed high concentration of total dissolved solids (TDS) (10350 mg /L) which exceeded the BIS limit of 2000 mg /L. Total suspended solids (TSS) denote the suspended pollutants present in the waste water which are contributed by fiber scrap and undissolved raw materials in the production process [38].Chloride content of the effluent was extremely high (891 mg /L) which is an index of the surface pollution level. Based on BOD (1100 mg /L) and COD (4520 mg /L) values, the effluent could be classified as high strength effluent indicating the risk posed to the aquatic ecosystem. BOD/ COD ratio, also called the "Biodegradability index" (B.I.) has been commonly used as an indicator for biodegradation capacity and is the cut-off point between biodegradable and non-biodegradable waste [33,39,40]. A BOD/ COD ratio of > 0.6 indicates fair biodegradability of the waste, while BOD/COD <0.3, inhibits biodegradation and the effluent cannot be treated biologically, because the wastewater inhibits the metabolic activity of

bacterial seed due to their refractory properties [41,42]. BOD/COD of 0.24 in the present study indicated that the effluent was not amenable to biological treatment and required more potent method for its management.

Table 2. Physico-chemical characteristics of textile effluent before and after biosorption by *R. arrizus*

Sr. No.	Test parameter	Textile effluent		BIS tolerance limits (1981)
		Before biosorption	After biosorption	
1	Color(ADMI units)	Dark bluish purple colored (65320)	Colorless with a tinge of pale yellow (454.4)	≤150
2	Turbidity	Highly turbid	Slightly Turbid	NA
3	pH	11.23	2.44	5.5-9.0
4	Odor	Foul	Unobjectionable	Unobjectionable
5	Chlorides (mg /L)	891	95	500
6	Total dissolved solids (mg /L)	10350	1620	2000
7	Total suspended solids (mg /L)	150	60	100
8	Total solids (mg /L)	10500	1680	2100
9	Chemical oxygen demand (mg /L)	4520	463	250
10	Biochemical oxygen demand (mg /L)	1100	150	350

Ref: Bureau of Indian Standards IS 2490 (1981).

2. Optimization of significant variables using Central Composite Design (CCD)

CCD was employed with the particular combinations of four independent variables (pH, biosorbent dosage, speed of agitation and contact time) at five levels (- 2, -1, 0, +1, +2). The design matrix of 30 experimental runs along with the experimental results and the results of theoretically predicted responses (using the model equation) are shown in Table 3.

Table 3. Input variables and experimental design matrix for percent decolorization of textile effluent by *R. arrhizus*

Sr. No.	Variables				% Sorption	
	pH	Biosorbent Dosage	Speed of Agitation	Contact Time	Actual	Predicted
1	1	-1	-1	1	98.46	98.23
2	1	-1	1	-1	99.60	99.74
3	-1	1	-1	1	93.29	93.63
4	-1	1	1	1	92.05	92.13
5	1	1	-1	1	98.14	98.03
6	-1	-1	1	-1	98.29	98.20
7	1	-1	1	1	98.94	98.88
8	-1	-1	-1	-1	98.44	98.56
9	1	1	1	-1	98.76	98.41
10	-1	1	1	-1	95.12	95.42
11	1	-1	-1	-1	96.28	96.23

12	1	1	1	1	97.19	97.13
13	-1	1	-1	-1	95.70	95.79
14	-1	-1	-1	1	98.18	98.56
15	-1	-1	1	1	98.58	98.61
16	1	1	-1	-1	98.14	98.18
17	0	0	0	-2	99.30	99.34
18	0	0	0	2	98.20	98.06
19	-2	0	0	0	97.38	96.68
20	2	0	0	0	98.76	99.35
21	0	2	0	0	94.78	94.66
22	0	0	-2	0	98.53	98.28
23	0	-2	0	0	99.18	99.19
24	0	0	2	0	98.42	98.56
25	0	0	0	0	98.40	98.38
26	0	0	0	0	98.58	98.38
27	0	0	0	0	98.58	98.38
28	0	0	0	0	98.38	98.38
29	0	0	0	0	98.96	99.17
30	0	0	0	0	98.96	99.17

R^2=0.9640 R^2adj= 0.9493 R^2pred= 0.9255

The percent sorption ranged from 92.05% to 99.60%. The highest percent sorption of 99.60 % was obtained in run No. 2 using following conditions: pH 2.5, biosorbent dosage 3 g /L, speed of agitation 85 rpm and contact time 60 min. Multiple regression analysis was used to analyze the data and to obtain empirical model for the best responses and thus second-order polynomial equation was obtained (Eq. 23):

$$Y=98.77+(0.73*X_1)+(-1.15*X_2)+(0.12*X_3)+(-0.37*X_4)+X_1*(X_2*1.09)+X_1*(X_3*0.28)+X_1*(X_4*-0.37)+X_3*(X_2*-0.46)+X_3*(X_4*-0.32)+X_3*(X_4*-0.47)+X_1*(X_1*-0.28)+X_2*(X_2*-0.56)+X_3*(X_3*-0.18)+X_4*(X_4*-0.11) \quad (22)$$

The mathematical expression of the relationships between the independent variables and dependent response are given in terms of uncoded factors. Apart from the linear effect of the parameters on the dye removal, the RSM gives an insight into the quadratic and interaction effects of the parameters. These analyses are done by means of Fisher's *F* test and Student's *t* test. Student's *t* test is used to determine the significance of the regression coefficients of the parameters. The regression coefficient and the *t* and *P* values for all the linear, quadratic, and interaction effects of the parameters were calculated to study the contribution of each variable towards biosorption as shown in Table 4.

Table 4. Estimated regression coefficients for percent biosorption of dyes in the textile effluent

Term	Coefficient	SE Coefficient	*t*- Statistics	*P*-Value
Constant	98.7707	0.2054	480.7	0
Block	-0.3822	0.0962	-3.97	0.001
pH	0.7297	0.1014	7.191	0
Biosorbent dosage	-1.1545	0.1014	-11.377	0
Speed of agitation	0.1165	0.1014	1.148	0.270
Contact time	-0.3675	0.1014	-3.621	0.003

pH*pH	-0.2833	0.0949	-2.984	0.010
Biosorbent dosage*Biosorbent dosage	-0.5558	0.0949	-5.855	0
Speed of agitation* Speed of agitation	-0.1822	0.0949	-1.919	0.076
Contact time*Contact time	-0.1119	0.0949	-1.179	0.258
pH*Biosorbent dosage	1.0871	0.1242	8.747	0
pH*Speed of agitation	0.2814	0.1242	2.265	0.040
pH*Contact time	0.3704	0.1242	2.981	0.010
Speed of agitation*Biosorbent dosage	-0.4578	0.1242	-3.684	0.002
Speed of agitation*Contact time	-0.3164	0.1242	-2.546	0.023
Biosorbent dosage*Contact time	-0.4681	0.1242	-3.766	0.002

The variable with the highest *t*- value is considered to be the best and is ranked one. Based on the *t*- values, the variables were ranked in the following order: biosorbent dosage, pH, contact time and speed of agitation. The variable with the large coefficient, either positive or negative, indicates a large impact on the response. A positive sign of the effect of the tested variable indicates its greater influence on biosorption at a high level and a negative effect indicates greater influence of the variable at a low level. Analysis of the regression coefficients of four variables showed that pH and speed of agitation had a positive effect on biosorption while biosorbent dosage and contact time had a negative effect on biosorption. The corresponding probability values (*P* values) point towards the significance of each of the coefficient [43]. From very small P values ($P < 0.05$) for all the linear effects (except for speed of agitation), quadratic effects (except for speed of agitation and contact time), as well as all the interaction effects, it can be said that the coefficients were highly significant. These measures indicated that the accuracy and general ability of the polynomial model was good and that analysis of the response trends using the model was reasonable.

The statistical implication of the ratio of the mean square variation due to the regression and mean square residual error was also tested using analysis of variance (ANOVA) as shown in Table 5. The ANOVA of the quadratic regression model demonstrated that the model was highly important, as was evident from the low *P*-value of the Fisher's *F*-test (F_{model}, 25.71) [($P_{model}>F$) = 0.000]. The lower calculated $F_{14,14}$-value (8.99) than the tabulated *F*-value even at the 0.0001 confidence level showed a statistically unimportant lack of fit. The model was found to be adequate for prediction within the range of variables employed.

Table 5.ANOVA for response surface quadratic model

Source	*DF*	*Seq SS*	*Adj SS*	*Adj MS*	*F*-value	*P*-value
Blocks	1	3.8949	3.8949	3.8949	15.76	0.001
Regression	14	88.97	88.97	6.355	25.71	0.000
Linear	4	48.3325	48.3325	12.0831	48.89	0.000
Square	4	9.8063	9.8063	2.5162	9.92	0.001
Interaction	6	30.8311	30.8311	5.1385	20.79	0.000
Residual Error	14	3.4599	3.4599	0.2471		
Lack-of-Fit	10	3.4236	3.4236	0.3424	37.72	0.002
Pure Error	4	0.0363	0.0363	0.0091		
Total	29	96.3247				

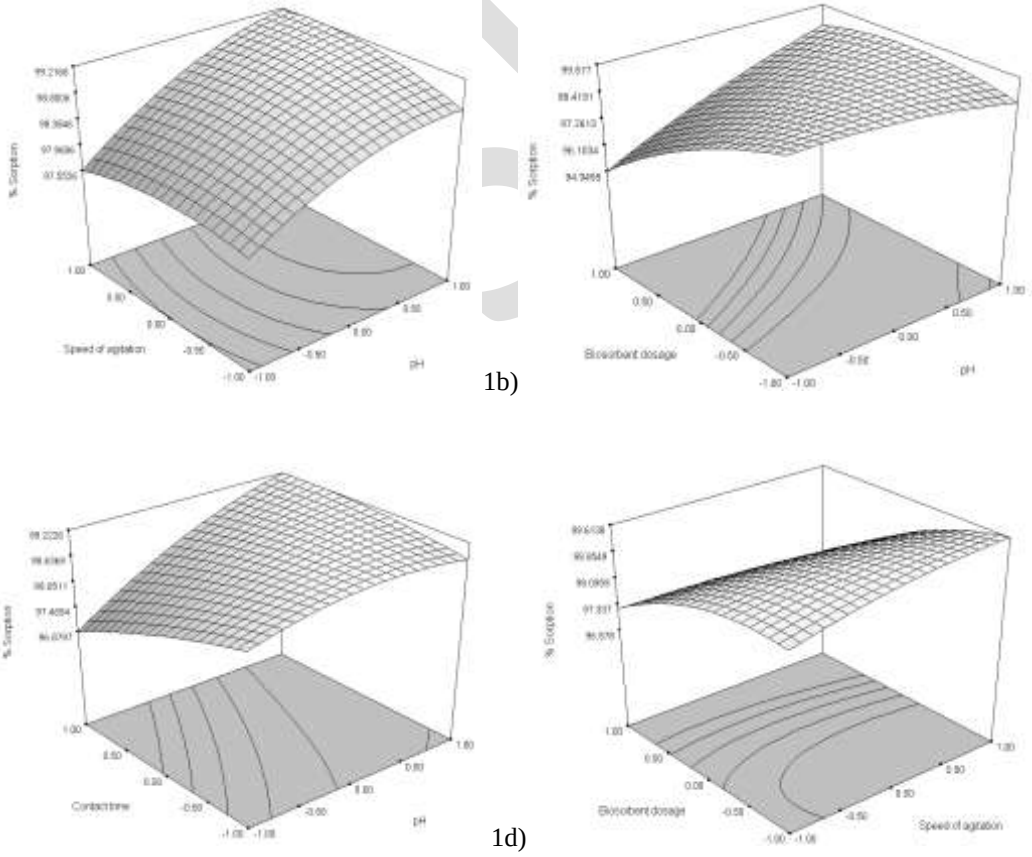
DF: Degree of freedom; *SS*: Sum of squares; *MS*: Mean sum of square; CV=0.36%;

The values of R^2 and *Adj*-R^2 were close to 1.0, which advocates a high correlation between the observed values and the predicted values. It indicates the efficiency of the regression model in providing an excellent explanation of the relationship between the independent variables and the response [44]. The R^2 also indicated that only 3.60 % of the

total variations could not be explained by the model. The pred-R^2 of 0.9493 was in reasonable agreement with *adj*-R^2 of 0.9255 indicating that the model is significant. The adjusted coefficient of determination represents the proportion of the variation in the response explained by the regression model. It is thus envisaged that Eq. 23 can capture 96.4% of the variation in the measured values of percent decolorization as a function of four independent variables within the ranges considered in the present study. The value of CV (0.36%) demonstrated that the performed experiments were reliable. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 29.197obtained in this study indicated an adequate signal. Therefore, this model can be used to navigate the design space. The ANOVA thus indicated that the second-order polynomial model was highly significant and adequate to represent the actual relationship between the response (percent decolorization) and variables, with $P < 0.0001$ and a high value of the coefficient of determination (96.94%).

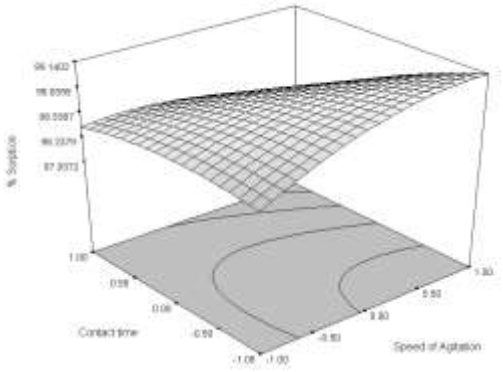
The graphical representations of the regression model, called the 3D surface plots were obtained for the estimated responses to investigate the interactive effects of variables on the dye removal from the effluent and to determine the optimum level of each variable for maximum response (percent decolorization) (Fig 1 (a-f)). A surface plot can be used to explore the relationship between three variables. Here, each response surface plot represented the effect of two independent variables varying in the factorial part of the experimental space (between -1 and +1), with the other independent variable set at its central level. These surface plots provide a method to predict the biosorption efficiency for different values of the tested variables.

Fig.1.Three-dimensional response surface plots of dye removal from effluent by dead fungal biomass of *R. arrhizus* showing variable interactions between a) pH*Speed of agitation b) pH*Biosorbent dosage c) pH*Contact time d) Speed of agitation*Biosorbent dosage e) Speed of agitation*Contact time f) Biosorbent dosage*Contact time

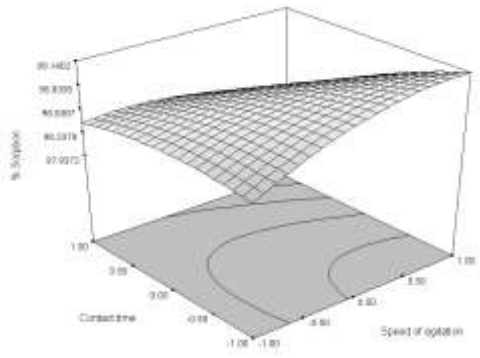


1a) 1b)

1c) 1d)



1e)



1f)

As seen in Fig. 1a–f, each response surface yield showed a clear peak suggesting that the optimum point was inside the design boundary [28]. The surface plot based on independent variables, i.e., pH (X_1) and speed of agitation (X_3), while the other independent variables biosorbent dosage (X_2) and contact time (X_4) were kept at zero level is shown in Fig. 1a. The figure revealed an interaction behavior with a positive main effect of both pH and speed of agitation, where the contribution of pH was prominent compared to that of speed of agitation. When the pH was increased from 1.5 to 2.5, the percent biosorption increased from 96.37 % to 98.27%, while only a slight increase was achieved when the speed of agitation was raised from 75 rpm to85 rpm. This indicated that high values of pH in the acidic range employed and speed of agitation yielded high biosorption capacities. Agitation promotes adequate contact between the dye ions in the solution and the binding sites on the biomass. It is known that, external film diffusion can influence the rate of biosorption process. With appropriate agitation, this mass transfer resistance thickness and the liquid boundary layer around the biosorbent particles are minimized [45-47]. This enhances the diffusion rate of a solute from the bulk liquid to the liquid boundary layer surrounding biosorbent particles thereby promoting effective transfer of dye ions to the biosorbent sites [48,49].

The pH of the dye solution was also involved in a two-way interaction with biosorbent dosage as well as contact time (Fig. 1b and 1c). The profile shown in Fig. 1b presents an interaction between pH and biosorbent dosage with the other independent variables kept at zero level. It suggested that percent biosorption increased when the pH of the system increased from 1.5 to 2.5 and biosorbent dosage decreased from 5 g /L to 3 g /L. The interaction between the dye molecules and the biosorbent is basically a collective result of charges on the dye molecules and the surface of the biosorbent [50].The dyes present in the effluent employed in this study were typically monoazo dyes containing various types of reactive groups such as vinylsulphone and monochloro- *s* triazine that are known to interact with the active groups on the cell surface of the fungal cell wall. The hexosamines-chitin and chitosan constituting approximately 24–40% of the cell dry weight of *Rhizopus* serve as a matrix of various functional groups like amine R–NH_2 (amino acids, proteins, glycoproteins, etc.), carboxylic (fatty acids, lipopolysaccharides, etc.), phosphate and sulfonate which take part in binding of dye ions [51].

Solution pH other than determining the surface charge of the biosorbent also determines the degree of ionization and speciation of the adsorbate, which affects the adsorption of dyes. Reactive dyes are anionic dyes because of the negative electrical structure of their azo based chromophore group and release colored dye anions in solution. At low pH of the solution, more protons are available to protonate the amino groups of chitosan molecules thereby forming positively charged–NH_3^+ groups that provide cationic anchor points with which the dye anions interact via ionic bonding. The chemical affinity between the positive charge of the protonated amino group ($–NH_3^+$) of biomass and negative charges in the structures of anionic dyes ($-SO^{3-}$) weakens the resistance of the boundary layer surrounding the biosorbent leading to more sorption under acidic conditions by electrostatic attraction [52,53]. Higher pH values (> 3), lead to deprotonation of the surface groups contributing to the decreased removal of dyes due to the electrostatic repulsion [54,55]. A similar trend for binding of reactive and acid dyes by fungus *R. arrhizus* and alga *Enteromorpha prolifera* has shown maximum values in the range pH 2–3 with a sharp drop off at higher values [28,53, 57]. Various reasons have been suggested to explain the reduced uptake capacity at increasing biomass including electrostatic interactions, overlapping or aggregation of adsorption sites resulting in a decrease in the total adsorbent surface area, interference between binding sites and reduced mixing at higher biomass densities [58-60]. Biosorbent dosage also exhibited interaction with speed of agitation (Fig. 1d) and contact time (Fig. 1f). The highest percent biosorption of 99.60 % was achieved with contact time of 60 min.

3. Validation of the model

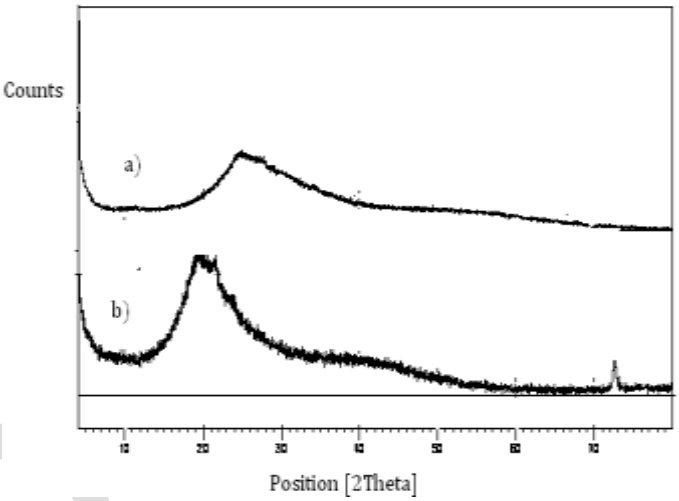
To assess the prediction capability of the model developed, several validation experiments were carried out in the region of experimentation surrounded by the factorial points (x_i varying between -1 and +1) [61]. The special feature of the RSM tool, 'Point prediction' was used to find optimum value of the combination of the four factors for the maximum biosorption. The observed values of percent decolorization (99.60%) were in good agreement with the predicted values (99.24 %) and fell into the 95% prediction intervals, further validating the model presented above. The predicted optimal conditions were as follows: pH 2.5, biosorbent dosage 3.7 g /L, speed of agitation 85 rpm, and contact time 69 min. The results revealed the efficiency of the biosorbent in removing not only color but other pollutants in the textile effluent as well. The biosorbent achieved a high degree of removal of color (99.60%), chloride (88.94%), TDS (84.35%), TSS (60%), TS (84%), COD (89.76%), BOD (86.36%). Thus, the dead biomass of *R. arrhizus* showed potential to be used as an efficient biosorbent for the treatment of biologically refractory textile effluent.

4. Mechanism of biosorption of dyes in the textile effluent by R. arrhizus

The relatively short contact time, necessary for achieving equilibrium conditions (an hour as seen in this study) is considered as an initial indication that the adsorption of dyes on *R. arrhizus* is a chemically controlled process, rather than a diffusion controlled one [28].

The mechanism of adsorption of dyes in the effluent by *R. arrhizus* was elucidated on the basis of BET, XRD and FTIR analysis. The BET analysis revealed that the biomass had a low surface area (0.618m^2/g) and showed a mesoporous structure (pore diameter=64.789Å). Dubinin (1967) suggested that mesopores are instrumental in the transport of adsorbate molecules to the micropores and the pore size distribution is of more importance than the surface area. High biosorption values achieved in the present investigation despite a low surface area could also be attributed to the mesoporous structure of the biosorbent as revealed by the BET analysis. X-ray diffraction technique is a powerful tool to analyze the crystalline nature of the materials. Adsorption of dye may lead to change in molecular and crystalline structure of the adsorbent. The resulting changes provide a valuable information regarding adsorption reaction while elucidating the molecular and crystalline structure of the adsorbent [64]. The XRD pattern of *R. arrhizus* biosorbent revealed poorly resolved peaks indicating a predominance of amorphous material indicating its suitability for biosorption. The broad peaks were obtained at 2θ= 19.7572, 21.4273, 24.0244 and a small sharp peak at 2θ= 72.6714 with *d* spacing 4.49365, 4.14703, 3.70429 and 1.30113, respectively. Presence of broad peaks can be attributed to a heterogeneous and complex matrix composed of several substances, including proteins, lipids, carbohydrates etc. The XRD patterns of the dye-laden biomass displayed more amorphous characters, suggesting that the dye molecules were adsorbed on the surface mostly by chemisorption causing alteration of the structure of the biomass, as a result of the adsorption reaction (Fig. 4) [61].

Fig.4. XRD pattern of (a) biosorbent and (b) wastewater loaded biosorbent

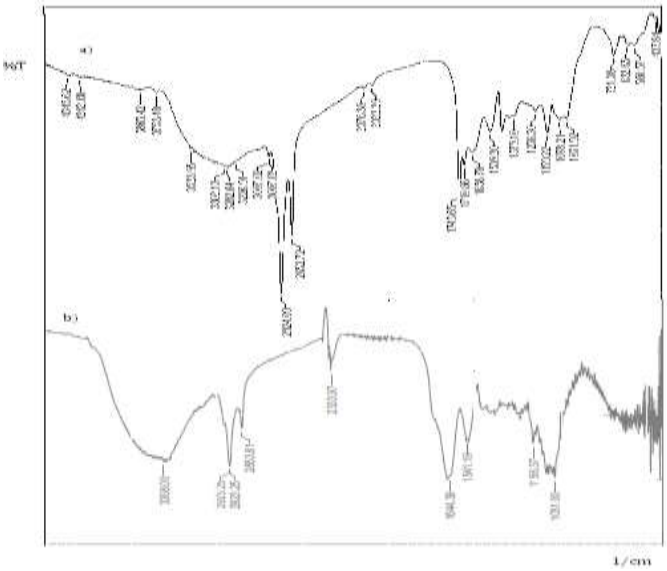


The FTIR spectral analysis is important in identifying the characteristic functional groups, which are responsible for biosorption of dye molecules. A closer insight into the biomass surface properties was obtained by comparing the FTIR spectra of dye effluent loaded biomass of *R. arrhizus*, with that of the unloaded biomass in the range of 400–4000 cm^{-1}. The infrared spectrum of the biomasses displayed characteristic bands of proteins, lipids, polymeric compounds indicating the complex and heterogeneous nature of the biomass [62,63]. Peaks between 3500 to 3200 cm^{-1} represent the presence of –OH groups of alcohols and phenols and primary, secondary amines or amide –N-H groups, while peaks between 3300 to 2500 cm^{-1} indicate O-H stretch of carboxylic acids. Peak positions between the wave number range of 2000 cm^{-1} to 500 cm^{-1}, indicate the vibrational modes that are specific to the type of polysaccharides and glycosidic linkages. Peaks in this range denote the presence of aldehyde functional groups which are actively involved in biosorption. Amide I bands (1700cm^{-1}-1600cm^{-1}) due to C=O stretching vibrations of peptide bond provide insight into the secondary structure of proteins [124]. Similar peaks (1658.78 cm^{-1}) were obtained in the biosorbent used in this study. Peaks between 910-665 cm^{-1} represent N-H wag of primary and secondary amines.

The spectrum displayed the absorption peaks at 3523.95–3226.91, 1743.65, 1710.86, 1658.78, and 1238.3 cm^{-1}, corresponding to carboxyl groups. Shifts in peaks corresponding to carboxylic groups (1658.78 to 1644.39cm^{-1}) and disappearance of peaks at 3523.95, 3302.13, 3282.84, 3226.91, 1743.65, 1710.86, 1238.3 cm^{-1} after dye biosorption indicates their involvement. Disappearance of peaks at 3226.91 and 3302.13 $cm^{-1}$and shifting of peaks at 1539.2 and

$1379.1cm^{-1}$ indicates role played by amine groups in the binding of dyes in the effluent. Chitin carries one linear amino group per glucose unit and thus exhibits a high dye uptake capacity. The amino group has an electron pair available for coordination and behaves like a strong Lewis base. Disappearance of peak at 3282.84 cm^{-1} indicated involvement of hydroxyl groups of the polysaccharides, which are the other available Lewis bases in the cell wall [51]. The contribution by C-O stretching vibrations,-NH bending, C-O-C linkage, in binding of the dyes was revealed by shifting of bands at 1539.2, 1159.22 and 2852.72 cm^{-1}. The involvement of some phenolic groups in the biosorption was apparent by disappearance of peak at 1379.10 cm^{-1} after binding of dyes [65]. Thus, FTIR spectra elucidated the role played by amine, hydroxyl, carboxyl and phenolic groups in biosorption of dyes (Fig. 5).

Fig. 5.FTIR pattern of (a) biosorbent and (b) wastewater loaded biosorbent



Overall, the sorption process can be described as follows: Reactive dyes are first adsorbed on the surface of *R. sarrhizus* by chemisorption as indicated by conformation to Langmuir, Fruindlich and Temkin isotherm models. The adsorption of dye takes place probably via electrostatic interaction until the surface functional sites are fully occupied; thereafter dye molecules diffuse into the pores of the biosorbent layers for further interactions [19]. The low BET specific surface area of the biosorbent ($0.618 m^2/g$) implied that the mechanism was mainly controlled by surface adsorption. A greater boundary layer effect shown by intra-particle diffusion model also suggested a greater contribution of the surface adsorption in the rate limiting step. Similar results were reported by Uzun (2006) [66]. This suggests that the biosorption system studied belonged to the second-order kinetic model, based on the assumption that the rate-limiting step may be chemical adsorption [67].

IV. CONCLUSION

In the current investigation, fungal biosorbent made from the dead biomass of *R. arrhizus* was used successfully for the sorption of reactive dyes from the textile effluent using statistical optimization strategy. The RSM approach allowed assessment of the influence of the main process variables on biosorption of the dyes and provided useful indications on the optimal set of operating conditions to be used. The interactive effects of four independent factors: initial pH of solution, biosorbent dosage, speed of agitation and contact time on biosorption were studied using CCD. A very high sorption rate of 99.60 % of dyes from wastewater was obtained by fungus *R. arrhizus* under the optimum conditions i.e., pH 2.5, biosorbent dosage 3 g/L, speed of agitation 85 rpm, contact time 60 min. A reduced quadratic model was obtained for predicting the percent decolorization. The excellent correlation between predicted and experimental values confirmed the validity and practicability of this statistical optimum strategy. Thus, the use of RSM approach in combination with a mechanistic model can be a useful tool for an improved analysis of dye biosorption data and a more effective design of experiments. In order to evaluate the economic feasibility of the biosorbent for commercial application besides providing a view on the adsorption mechanism, isotherm and kinetic studies were carried out. The fitting of experimental data to Langmuir, Fruindlich and Temkin isotherm models suggested that the biosorption process was a monolayer capacitive sorption process in which heterogeneous surface conditions co-existed. Langmuir isotherm best described the process of biosorption. The pseudo second-order followed by Elovich equation provided a good fitting to the experimental data points suggesting that the adsorption system studied was based on the assumption that the rate-limiting step may be chemical sorption or chemisorption. The BET, XRD and FTIR analysis further helped in understanding the mechanism of biosorption. The study indicated that the fungal biosorbent is an effective and economical alternative for the removal of the dyes, total solids, chlorides, BOD and COD from the textile effluents. The results obtained in this work provide a basis for the use of biosorption as an efficient treatment method for the textile wastewater.

ACKNOWLEDGMENTS

The authors thank S.P. Consultants, Mumbai for providing facilities for BET analysis.

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