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Response Surface Methodology and Central Composite Design Analysis of Biosorption of Reactive Anthraquinone Dyes by *Citrus sinensis* Waste Biomass

MAHWISH ASGHER, HAQ NAWAZ BHATTI*, IJAZ AHMAD BHATTI and MUNIR AHMAD SHEIKH
Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan
Fax: (92)(41)9200764 ; E-mail: hnbhatti2005@yahoo.com

The objective of present research work is to optimize the physicochemical parameters influencing the biosorption of reactive anthraquinone dyes by *Citrus sinensis* biosorbent using central composite design under response surface methodological approach. The quadratic model explained the biosorption data with high regression coefficients, R^2 0.9882 for reactive blue 19 and 0.9999 for reactive blue 49. Interactive effect of pH, biosorbent dose and dye concentration was found significant and sorption capacity was optimum at acidic pH range, smaller biosorbent dose and higher dye concentrations. The 2-factor interaction of pH, biosorbent dose and dye concentration significantly influenced the sorption potential of biosorbent.

Key Words: *Citrus sinensis*, Reactive anthraquinone dyes, Biosorption, Response surface methodology, Central composite design.

INTRODUCTION

Recently, reactive dyes have found wide applications in textile industries to colour the cellulosic fibers regarding desired characteristics of bright colour, water fastness and easy application techniques[1,2]. These dyes consist of azo or anthraquinone chromophore along with different types of reactive groups which may be a heterocycle such as monochlorotriazine, dichlortiazine or it may be an activated double bond like vinyl sulphone[3]. These dyes differ from other dyes in that they are first adsorbed on cellulose and then react with the fibers by forming covalent bond. However, 10-50 % of the dye is left in the dye bath giving a highly coloured effluent causing serious types of problems in the environment[4]. Reactive dyes being chemically stable and having low biodegradability tend to pass through conventional plants without treatment so their removal is of great importance[5,6].

Conventional technologies such as coagulation/flocculation, advanced oxidation processes, ozonization, membrane filtration and biological treatment are rather expensive and result in accumulation of large sludge with obvious disposal problems[7,8]. Biosorption has been recognized as an alternative technology to remove dye molecules from dilute aqueous solutions using inactive and dead biomass[9]. Biosorption is generally rapid process and uses cheaper materials such as waste biomass from agriculture and industry being abundant in nature, inexpensive and require little processing[10,11].

Conventionally optimization of experimental parameters has been carried out by checking the influence of one variable at a time on an experimental response keeping the other variables at constant level. However this procedure may suffer with disadvantages such as large experimental size resulting in increased experimental time and expenses due to greater consumption of reagents and materials. Moreover the interactive effects among variables are not considered. This problem can be solved by using multivariate statistic techniques of which most relevant multivariate techniques used in analytical optimization is response surface methodology[12].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques helpful for evaluating the effects of several independent variables and their interactions on the response[13]. Response surface methodology has an important application in the process design and optimization of independent variables and interactive effects among the variables and, eventually, it depicts the overall effects of the parameters on the process[14]. The application of experimental design and response surface methodology (RSM) in textile effluent treatment process may enhance extent of decolourization and reduce process variability, time and overall costs[15]. The most popular response surface method is the central composite design that consists of two level factorial design points, axial or star points and center points.

The objective of this study is to optimize and evaluate the interactive effect of initial dye concentration, pH and amount of biosorbent on biosorption of reactive dyes using *Citrus sinensis* waste biomass by means of response surface methodology.

EXPERIMENTAL

Batch biosorption studies: The fresh fruits of *Citrus sinensis* were collected from local market of Faisalabad-Pakistan. After extraction of juice, the waste biomass was washed with distilled water to remove dust, dried in sunlight and crushed to fine powder using grinding mill. The sample was sieved to get uniform fraction of particle size < 0.25 mm.

The reactive dyes consisting of anthraquinone chromophore; reactive blue 19 (λ_{max} 595 nm) and reactive blue 49 (λ_{max} 595 nm), were obtained from the commercial market. Figs. 1 and 2 designate the chemical structures of reactive blue 19 and reactive blue 49, respectively. Reactive groups are monochlorotriazine in reactive blue 49 and vinyl sulphone in reactive blue 19. A stock solution of reactive dye (1000 mg/L) was prepared by dissolving 1g of dye in 1 L of distilled water. Necessary dilutions were carried out from the stock solution to prepare solutions in the range of concentrations 50-300 mg/L. The initial pH of each solution was adjusted to the required value with 1 N HCl and 1 N NaOH solutions before mixing the biosorbent suspension.

Batch sorption experiments were performed by shaking 250 mL Erlenmeyer flasks containing 50 mL of dye solution at 100 rpm for 1 h at 303 K. The concentration of dye solution before and after biosorption process was determined by spectrophotometer. The sorption capacity of biosorbent was calculated using the following equation:

$$q = (C_i - C_e)\ V/W \tag{1}$$

where q (mg/g) is the amount of dye sorbed by biomass, C_i and C_e (mg/L) are the initial and equilibrium liquid phase concentrations of the dye, respectively, V (L), the initial volume of dye solution and W (g), the weight of the biomass.

Experimental design and optimization: The Design Expert 7.1.6 (Stat-Ease) software was used for regression and graphical analysis of the obtained data. The central composite design (CCD) is the most frequently used under RSM design due to it's suitability to fit quadratic surface which usually works well for process optimization. The present research work employed the central composite design to investigate the effect of three variables *i.e.*, pH, biosorbent dose and dye concentration with five levels on the sorption capacity of *Citrus sinensis* biosorbent. A design of 15 experiments was constructed consisting of for four factorial points, 5 replicates at the central points and 6 axial or star points. The chosen independent variables used in this study were coded according to following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{2}$$

where x_i is the dimensionless coded value of the ith independent variable, X_0 is the value of X_i at the center point and ΔX is the step change value. Experimental ranges and levels of these variables are given in Table-1.

TABLE-1
EXPERIMENTAL RANGE AND LEVELS OF INDEPENDENT VARIABLES

	Range and levels (coded)				
	-2	-1	0	1	2
A: pH	1.50	1.75	2	2.25	2.50
B: Dye concentration (mg/L)	25	75	125	175	225
C: Biosorbent dose (mg/g)	0.05	0.08	0.1	0.13	0.15

The behaviour of response can be explained by the following empirical second-order polynomial model.

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii} x_j^2 + \sum_{i=1}^{k-1}\sum_{i=2}^{k}\beta_{ij} x_i x_j + \varepsilon \tag{3}$$

where Y is the predicted response, x_i, x_j,. . . , x_k are the input variables, which affect the response Y, $x^2{}_i$, $x^2{}_j$, . . . , $x^2{}_k$ are the square effects, x_ix_j, x_ix_k and x_jx_k are the interaction effects, β_0 is the intercept term, β_i (i = 1, 2,. . . , k) is the linear effect, β_{ii}

(i = 1, 2,. . . , k) is the squared effect, β_{ij} (i = 1, 2,. . . , k; j = 1, 2,. . . , k) is the interaction effect and ε is a random error[16-18]. The optimum values of the selected variables were obtained by solving the regression equation at desired values of the process responses as the optimization criteria. The data were subjected to analysis of variance and the coefficient of regression (R^2) was calculated to find out the goodness of fit of the model.

RESULTS AND DISCUSSION

Response surface modeling: The experimental design matrix derived from central composite design for three coded independent variables along with observed responses, q (mg/g), for reactive blue 19 and reactive blue 49 is presented in Table-2. The experimental results were evaluated and polynomial equations fitted to sorption data of reactive blue 19 (eqn. 4) and reactive blue 49 (eqn. 5) are given as follows:

$$Q\ (mg/g) = 17.84 - 1.72\ A + 2.86\ B - 1.54\ C - 0.27\ AB + 1.44\ AC - 1.09\ BC - 0.59\ A^2 - 1.06\ B^2 + 1.25\ C^2 \tag{4}$$

$$Q\ (mg/g) = 58.60 - 0.74A + 18.80\ B - 13.12\ C - 8.68\ AB - 2.62\ AC - 6.09\ BC - 8.79\ A^2 - 2.91\ B^2 + 1.86\ C^2 \tag{5}$$

where A, B and C are three independent variables. Significance of each coefficient in eqns. 1 and 2 was determined by Student's t-test and p-value.

TABLE-2
CENTRAL COMPOSITE DESIGN MATRIX FOR THREE CODED INDEPENDENT VARIABLES ALONG WITH OBSERVED RESPONSE, q (mg/g) OF *Citrus sinensis* BIOSORBENT FOR REACTIVE DYES

Run No.	A: pH	B: Dye Concentration	C: Biosorbent Dose	Reactive blue 19 Response (Y): q (mg/g)	Reactive blue 49 Response (Y): q (mg/g)
1	0	0	0	17.50	58.35
2	0	0	0	16.80	58.43
3	-1	1	1	18.12	60.12
4	-1	-1	-1	17.80	26.18
5	2	0	0	12.10	22.08
6	0	-2	0	7.94	9.49
7	1	1	-1	19.40	79.72
8	0	0	2	19.80	39.92
9	0	0	0	18.80	58.68
10	1	-1	1	14.01	27.99
11	0	0	-2	25.95	92.42
12	-2	0	0	18.96	25.03
13	0	0	0	18.50	58.85
14	0	0	0	17.70	58.92
15	0	2	0	19.40	84.69

Fitting of quadratic model: The statistical significance of the fitted quadratic model was determined by the analysis of variance (ANOVA). ANOVA is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameter of the model[19]. Results of analysis of variance of this model for biosorption of reactive blue 19 and reactive blue 49 are reported in Tables 3 and 4, respectively. These tables show the statistical significance of ratio of mean square due to regression and mean square residual error so the model can be used to navigate the design space. According to the ANOVA, F values are very large showing that model terms are significant and most of the variation in the response can be explained by the regression equation. Values of "prob > F" less than 0.0500 also indicate high significant regression at 95 % confidence level. The lack-of-fit term is statistically insignificant as desired. The fit of the model was checked by the determination of coefficient (R^2). The coefficient of determination for colour removal ($R^2 = 0.9882$ for reactive blue 19 and 0.9999 for reactive blue 49) defines that 99 % of the variation for removal of colour is explained by the independent variables and about 0.01 % of sample variation for colour removal is not explained by the model. The value of adjusted determination coefficient (adjusted $R^2 = 0.9668$ for reactive blue 19 and 0.9998 for reactive blue 49) is also high indicating high significance of the model. This reveals that the regression model explains the relationship between the independent variables and the response q (mg/g) very well. The plots of normal % probability versus studentized residuals are shown in Figs. 1 and 2 for reactive blue 19 and reactive blue 49, respectively. These figures indicate acceptability of model according to the assumptions of the analysis of variance (ANOVA) and there is also no problem with normality nor is any response transformation required[20]. The actual and the predicted biosorption capacity of *Citrus sinensis* biosorbent for reactive blue 19 and reactive blue 49 are shown in Figs. 3 and 4. These figures designate the goodness of fit of model to response data.

TABLE-3
ONE WAY ANOVA FOR RSM PARAMETERS FITTED TO POLYNOMIAL EQUATION FOR BIOSORPTION OF REACTIVE BLUE 19 BY *Citrus sinensis* BIOSORBENT
ONE WAY ANOVA FOR RSM PARAMETERS FITTED TO POLYNOMIAL EQUATION

Sources of variation	Sum of squares	Degree of freedom	Mean square	F value	p-value Probability > F
Model	219.83	9	24.43	46.33	< 0.0003*
Lack of fit	0.064	1	0.064	0.100	0.7678[a]
Pure error	2.57	4	0.64		
Residual	2.64	5	0.53		
Total	222.47	14			

$R^2 = 0.9882$; Adj $R^2 = 0.9668$ and coefficient of variance = 4.14; [a]Not significant.

*Values of “Probability > F” less than 0.05 indicate model terms are significant.

TABLE-4
ONE WAY ANOVA FOR RSM PARAMETERS FITTED TO POLYNOMIAL EQUATION FOR BIOSORPTION OF REACTIVE BLUE 49 BY *Citrus sinensis* BIOSORBENT

Sources of variation	Sum of squares	Degree of freedom	Mean square	F value	p-value Probability > F
Model	8551.59	9	950.18	7644.36	<0.0001*
Lack of fit	0.37	1	0.37	5.86	0.0727[a]
Pure error	0.25	4	0.063		
Residual	0.62	5	0.12		
Total	2451.05	14			

$R^2 = 0.9999$; Adj $R^2 = 0.9998$ and coefficient of variance = 0.70; [a]Not significant.
*Values of "Probability > F" less than 0.05 indicate model terms are significant.

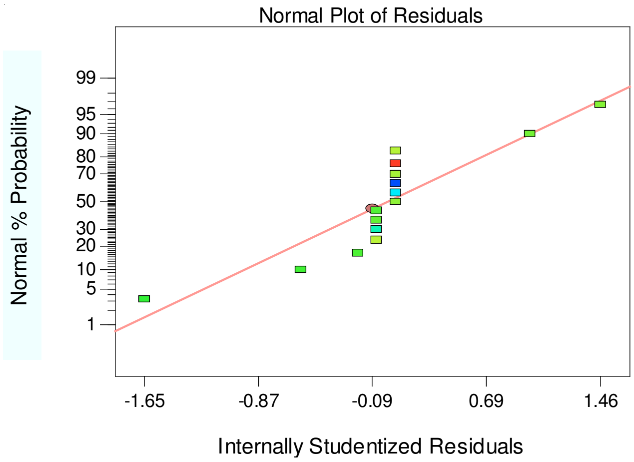

Fig. 1 The studentized residual and normal % probability plot of decolourization of reactive blue 19 by *Citrus sinensis* biosorbent

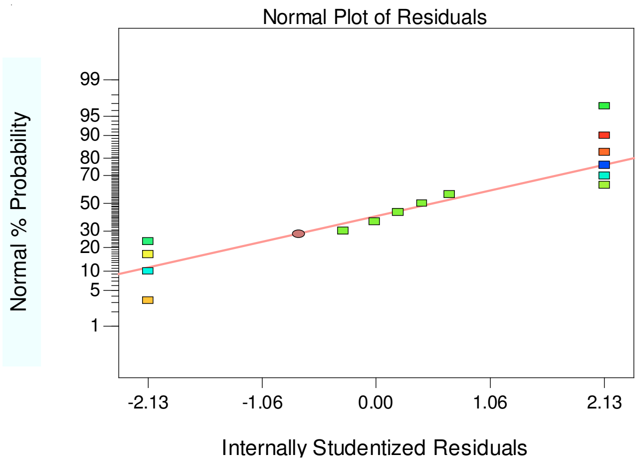

Fig. 2. The studentized residual and normal % probability plot of decolourization of reactive blue 49 by *Citrus sinensis* biosorbent

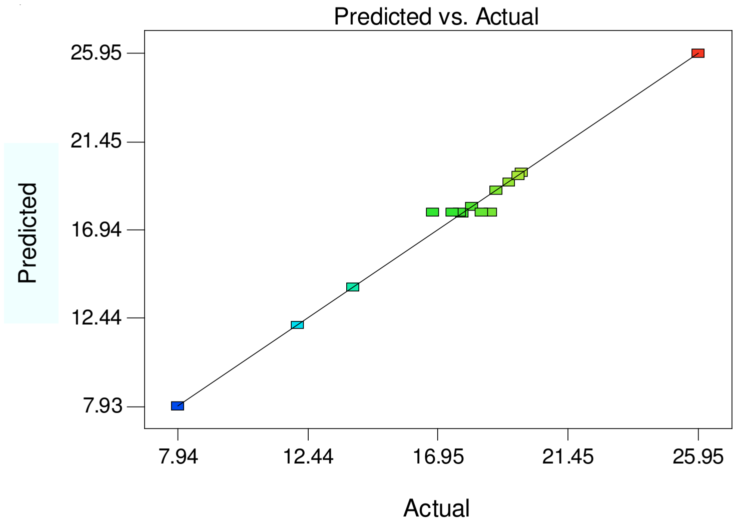

Fig. 3. Plot of predicted *versus* actual response, q mg/g, for decolourization of reactive blue 19 by *Citrus sinensis* biosorbent

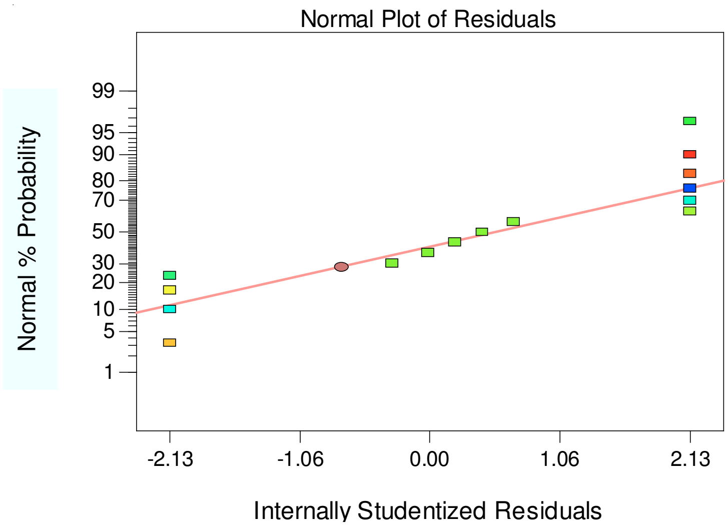

Fig. 4. Plot of predicted *versus* actual response, q mg/g, for decolourization of reactive blue 49 by *Citrus sinensis* biosorbent

Interactive effect of variables: Fig. 5 shows the interactive effect of pH and biosorbent dose on sorption capacity of *Citrus sinensis* biosorbent for reactive blue 19. The response surface shows a maximum at pH 1.5 and 0.50 g biosorbent dose. Further increase in pH and biosorbent dose result in decreased sorption capacity and interactive effect of pH and biosorbent dose was found to be significant. Fig. 6 designated the significant interaction of biosorbent dose and dye concentration on

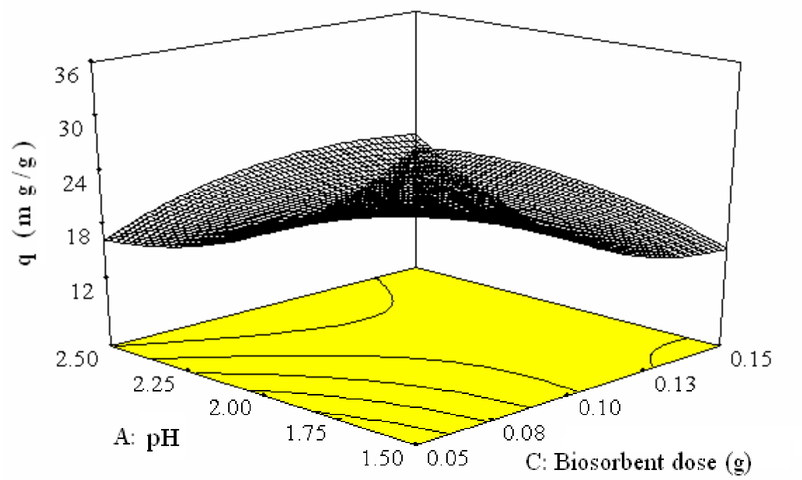

Fig. 5. Effect of interaction of pH and biosorbent dose on sorption capacity of *Citrus sinensis* biosorbent for reactive blue 19

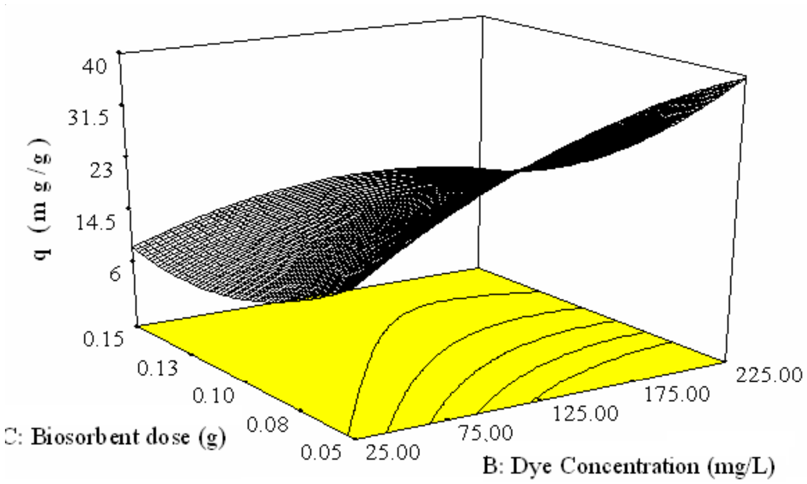

Fig. 6. Effect of interaction of biosorbent dose and dye concentration on sorption capacity of *Citrus sinensis* biosorbent for reactive blue 19

the sorption capacity of *Citrus sinensis* biosorbent for reactive blue 19. As initial dye concentration increased, sorption capacity also increased and decreased with increasing biosorbent dose. Fig. 7 indicates optimum sorption capacity at pH 2 and increased with increasing initial dye concentration. Interactive effect of both variables was found to be significant. Response surface presented in Fig. 8 shows significant interaction and a maximum at pH 2 and 0.05 g amount of biosorbent. Fig. 9 reveals significant interaction between biosorbent dose and dye concentration and also shows that optimum point is located outside the experimental region.

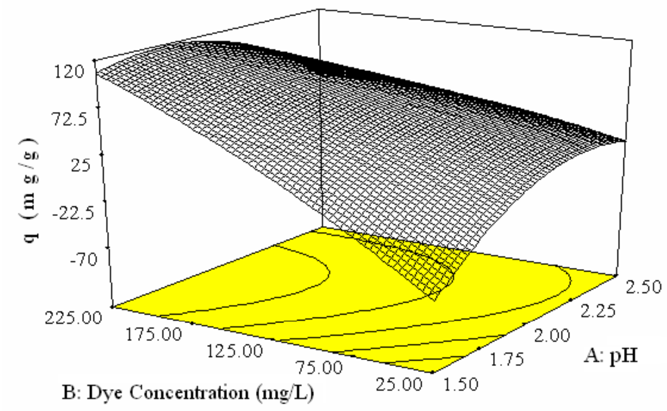

Fig. 7. Effect of interaction of pH and dye concentration on sorption capacity of *Citrus sinensis* biosorbent for reactive blue 49

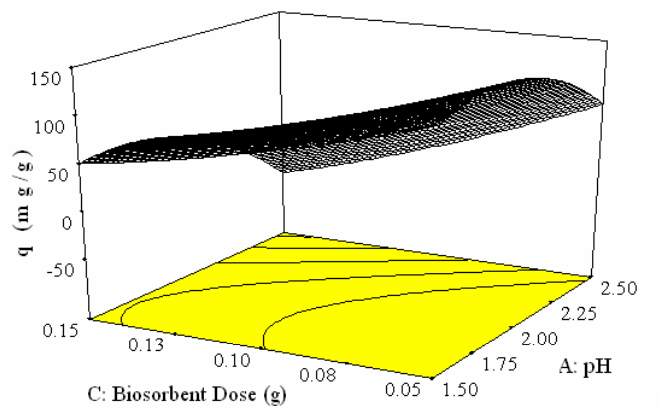

Fig. 8. Effect of interaction of pH and biosorbent dose on sorption capacity of *Citrus sinensis* biosorbent for reactive blue 49

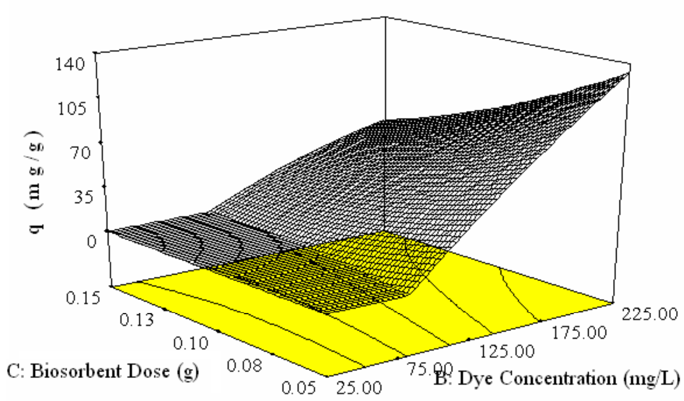

Fig. 9. Effect of interaction of biosorbent dose and dye concentration on sorption capacity of *Citrus sinensis* biosorbent for reactive blue 49

Conclusion

- The central composite design consisting of 4 factorial points, 6 axial points and 5 central points constructed under response surface methodology was found successful in explaining the sorption behaviour of reactive anthraquinone dyes from aqueous solution by *Citrus sinensis* biosorbent.
- The biosorbent prepared from *Citrus sinensis* waste biomass possessed high sorption potential for reactive blue 19 and reactive blue 49 under acidic conditions using smaller mounts of biosorbent for higher dye concentrations.
- The 2-factor interaction of pH, biosorbent dose and dye concentration significantly influenced the sorption potential of biosorbent.

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