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BIOSORPTION OF METHYLENE BLUE BY SPENT BIOMASS OF *Aspergillus terreus* USING RESPONSE SURFACE METHODOLOGY

V. SRIDEVI

Associate Professor, Dept. of Chemical Engineering, College of Engineering (A),
Andhra University, Visakhapatnam – 530 003.

ABSTRACT

Dye concentration of waste water can lead to a variety of problem. Biosorption has been proved to be a well established and cheap pollutant removal process among other purification techniques in this study our aim is to find the optimization of methylene blue by the spent biomass of *Aspergillus terreus* using response surface methodology. The spent biomass was received as a free sample from Krebs Bio chemicals Limited, which was the discarded biomass from their *Lovastatin* production plant. Box- Behnken design will be applied with 3 variables (dye concentration, adsorbent dosage and pH) in the chosen range so as to locate the optimal settings of the chosen variables. The spent biomass of *Aspergilus terreus* was found to be a potential low-cost biosorbent for the removal of methylene blue from water with a biosorption removal capacity of 94%. Box-Behnken design of RSM was proved to be a convenient tool for optimizing the biosorption process of dye removal, predicting a maximum methylene blue removal of 94% with the optimum process variables being found at 2.1 mg/100ml of initial methylene blue conc., 1.26 gm of adsorbent(*A. terreus*) dosage/100 ml solution, and a pH value of 7.9.

KEYWORDS: Biosorption, *Aspergillus terreus,* RSM, agitation time, methylene blue concentration, adsorbent dosage, pH, temperature.

V. SRIDEVI
Associate Professor, Dept. of Chemical Engineering,
College of Engineering (A), Andhra University, Visakhapatnam – 530 003.

**Corresponding author*

INTRODUCTION

Dyes have long been used in dyeing, paper and pulp, textiles, plastics, leather, cosmetics and food industries. Colour stuff discharged from these industries poses certain hazards and environmental problems[3]. These coloured compounds are not only aesthetically displeasing but also inhibiting sunlight penetration into the stream and affecting aquatic ecosystem. Dyes usually have complex aromatic molecular structures which make them more stable and difficult to biodegrade[2]. There are various conventional methods of removing dyes including coagulation and flocculation, oxidation or ozonation and membrane separation[6]. However, these methods are not widely used due to their high cost and economic disadvantage[1]. Chemical and electrochemical oxidations, coagulation are generally not feasible on large scale industries. In contrast, an adsorption technique is by far the most versatile and widely used.

RESPONSE SURFACE METHODOLOGY

Optimizing refers to improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it. Traditionally, optimization in Bioprocess Engineering has been carried out by monitoring the influence of one factor at a time on an experimental response. While only one parameter is changed, others are kept at a constant level[7]. This optimization technique is called ‘one-variable at a time’. The traditional ‘one-factor at a time’ technique used for optimizing a multivariable system is not only time consuming but also often easily misses the interactions between the components. Also, this method requires to carry out a large number of experiments to determine the optimum levels when the interactions are significant[5,8].

Choice of the experimental design

The simplest model which can be used in RSM is based on a linear function. For its application, it is necessary that the responses obtained are well fitted to the following equation[4]

$$y = \beta_0 \sum\nolimits_{i=1}^{k} \beta_i x_i + \varepsilon \qquad (2.1)$$

where k is the number of variables, β_0 is the constant term, β_i represents the coefficients of the linear parameters, x_i represents the variables, and ε is the residual associated to the experiments.

Therefore, the responses should not present any curvature. To evaluate curvature, a second-order model must be used. Two-level factorial designs are used in the estimation of first-order effects, but they fail when additional effects, such as second-order effects, are significant. So, a central point in two-level factorial designs can be used for evaluating curvature. The next level of the polynomial model should contain additional terms, which describe the interaction between the different experimental variables. This way, a model for a second-order interaction presents the following terms

$$y = \beta_0 + \sum\nolimits_{i=1}^{k} \beta_i x_i + \sum\nolimits_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (2.2)$$

where β_{ij} represents the coefficients of the interaction parameters.

In order to determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below

$$y = \beta_0 + \sum\nolimits_{i=1}^{k} \beta_i x_i + \sum\nolimits_{i=1}^{k} \beta_{ii} x_i^2 + \sum\nolimits_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (2.3)$$

Where β_{ii} represents the coefficients of the quadratic parameter.

To estimate the parameters in Eq. (2.3), the experimental design has to assure that all studied variables are carried out at in at least three factor levels. Thus, two modeling, symmetrical response surface designs are available. Among the more known second order symmetrical designs are the three-level factorial design, Box–Behnken design, central composite design, and Doehlert design. These symmetrical designs differ from one another with respect to their selection of experimental points, number of levels for variables, and number of runs and blocks.

MATERIALS AND METHODS

Preparation of Biosorbent

Aspergillus terreus is the waste biomass taken from the Krebs Bio chemicals, from their *Lovastatin* production unit. The biomass was dried using hot air oven at a temperature of 100˚c and then washed with ethanol in order to remove the unwanted waste. Subsequently, it was dried and powdered using motor and pestle and further sieved for its uniformity.

Preparation of dye solutions

Methylene blue, (MB) a cationic dye. The stock solutions at the desired concentrations were prepared with distilled water. The pH values of dye Solutions were adjusted with 0.001 N NaOH or HCl solutions using a pH meter.

RESULTS AND DISCUSSIONS

Effect of agitation time

Methylene blue solution is prepared with a concentration (5mg/100ml), adsorbent dosage (2g/100ml) and pH(8). Samples are withdrawn at 10min interval for estimating methylene blue concentration using spectrophotometer at the wavelength corresponding to maximum absorbance, λ_{max}, of 600nm. From the results shown in Figure & Table 1, it is evident that percentage removal of methylene blue remains constant from the time beyond 45 minutes. Hence all the subsequent runs were confined to this time limit.

Figure 1
Effect of agitation time on % removal of methylene blue

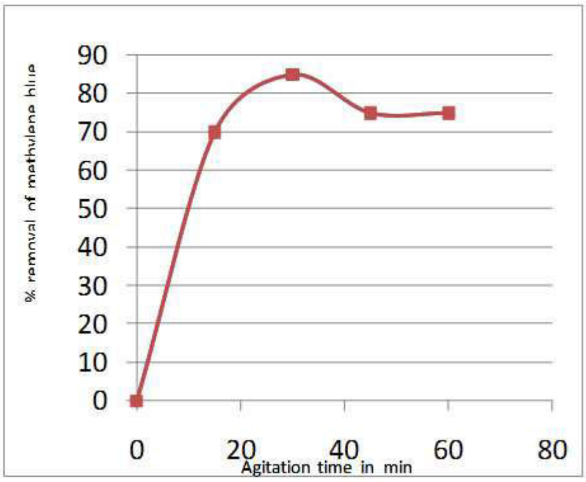

Table 1
Effect of agitation time on % removal of methylene blue

S.NO	Agitation time t, min	Final conc. Of methylene blue C_t, mg/100ml	% Removal of Methylene blue
1	0	0.040	0
2	15	0.012	70
3	30	0.011	72.5
4	45	0.010	75
5	60	0.010	75

Effect of methylene blue concentration

The methylene concentration varied from 1 to 6mg/100ml at a fixed adsorbent dosage of 1g/100ml with a pH value of 8. At lower initial concentration, percentage removal of methylene blue is higher. By this result the range of methylene blue concentration is chosen as 1-5mg/100ml for Box-Behnken Design (RSM).The results are shown in Figure & Table 2, from which it is obvious that at lower initial concentrations, percent removal of the dye is higher.

Figure 2
Effect of initial methylene blue concentration on % removal of methylene blue

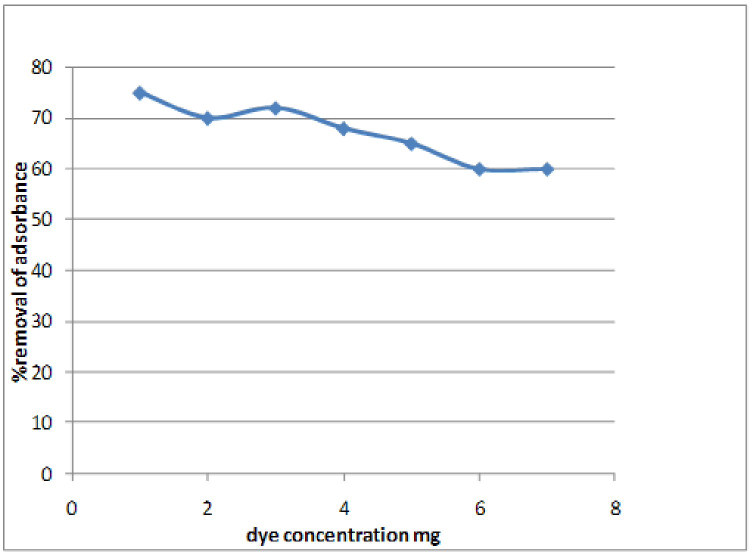

Table 2
Effect of Initial methylene blue concentration on % removal of MB

S.NO	Initial conc. of methylene blue in Aqueous solution C_0, mg/100ml	Equilibrium conc. of Methylene blue C_e, mg/100ml	% Removal of Methylene blue
1	1	0.009	77.75
2	2	0.014	71.4
3	3	0.016	70.3
4	4	0.017	70.2
5	5	0.022	65.7
6	6	0.026	61.7

Effect of adsorbent dosage

The effect of *Aspergillus terreus* dose on the biosorption of methylene blue was studied at a pH of 8 and dye concentration of 3 mg/100ml, by varying the biosorbant dosage in the range of 0.5-3g/100ml. The results are shown in Figure & Table 3. The percentage removal of methylene blue declined when adsorbent dosage was beyond 1gm. On the basis of this result, the range 1 to 2gm of adsorbent dosage is selected for RSM design.

Figure 3
Effect of adsorbent dosage on % removal of methylene blue

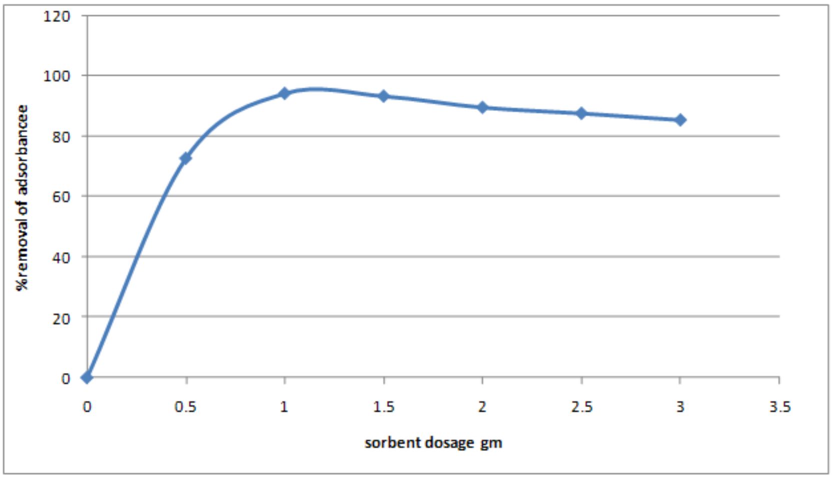

Table 3
Effect of biosorbent dosage on % removal of MB

S.NO	Biosorbent dosage m, g	Final conc. of methylene blue C_t, mg/100ml	% Removal of Metylene blue
1	0.5	0.009	72.72
2	1.0	0.003	94.23
3	1.5	0.004	93.33
4	2.0	0.008	89.60
5	2.5	0.011	87.64
6	3.0	0.015	85.43

Effect of pH

The uptake of methylene blue by *Aspergillus terreus* were studied in a range of 2-12 for pH by maintaining initial dye concentration of 3mg/100ml, biosorbent dosage of 1g/100ml solution, and a period of 45min, Maximum removal of methylene blue took place at a pH of 8.So 6 to 10 range is selected for the pH variable in the Box-Behnken design. The results are shown in Figure & Table 4.

Figure 4
Effect of pH on % removal of methylene blue

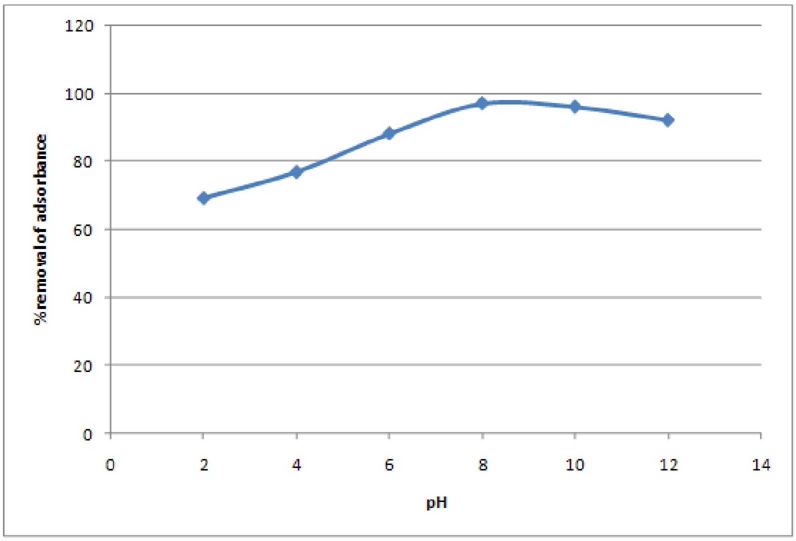

Table 4
Effect of pH on % removal of MB

S.NO	pH of the solution	Equilibrium conc. of Methylene blue C_e, mg/L	% Removal of Methylene blue
1	2	0.008	69.23
2	4	0.009	76.92
3	6	0.006	88.23
4	8	0.002	97.05
5	10	0.003	96.05
6	12	0.007	92.13

Box- Behnken Design

For optimizing a process with 3 variables at 3 levels, Box- Behnken design of RSM is widely used. On the basis of preliminary studies, the following range is fixed for the 3 chosen variables given in Table 5.

Table 5
Range of process variables for Box- Behnken design

Variable factors	Lower level (-1)	Middle level (0)	Upper level (+1)	Step change ΔX=difference between levels
Initial methylene blue conc. (mg/100ml)	1	3	5	2
***Aspergillus terreus* dosage (gm/100ml)**	1	1.5	2	0.5
Ph	6	8	10	2

The three chosen variables are coded according to the relation

$x = (X-X_0)/\Delta X$ ----- (1)

where x is coded variable, X is natural variable, X_0 is the middle point (zero level) and ΔX is the step change that represents the difference between the successive levels

Box- Behnken design consists of 15 experimental runs representing the different combination of factors that are represented in coded form: -1, 0, or +1. The runs: 13, 14, 15 are the replicates (repetitions) at the centre point and are used to estimate the experimental error. The relation between the responses (% dye uptake) with the factors is represented by the following quadratic equation

Y (% dye uptake) $= b_1+b_2x_1 +b_3x_2+b_4x_3+b_5x_1x_2+b_6x_1x_3+b_7x_2x_3+b_8x_1^2+b_9x_2^2+b_{10}x_3^2$..... (2)

The experimental values of response is calculated by

y (%dye uptake) $= 100\,(C_0-C_e)/C_e$…… (3)

and the corresponding values are listed in Table (6)

Table 6
Box - Behnken design

S.NO	Coded Values			Decoded values			% Removal of MB	
	X_1	X_2	X_3	MB x_1	*A.terreus* X_2	pH x_3	Y_{exp}	Y_{cal}
1	-1	-1	0	1	1	8	89	92.75
2	-1	1	0	1	2	8	77	81.75
3	1	-1	0	5	1	8	83	79.25
4	1	1	0	5	2	8	89	74.25
5	-1	0	-1	1	1.5	6	80	86.25
6	-1	0	1	1	1.5	10	80	77.25
7	1	0	-1	5	1.5	6	74	76.75
8	1	0	1	5	1.5	10	64	67.75
9	0	-1	-1	3	1	6	78	78.00
10	0	-1	1	3	1	10	81	76.00
11	0	1	-1	3	2	6	71	80.00
12	0	1	1	3	2	10	91	71.00
13	0	0	0	3	1.5	8	95	92.00
14	0	0	0	3	1.5	8	92	92.00
15	0	0	0	3	1.5	8	93	92.00

Using MATLAB program, 'regstats', the following equation is fitted

$$y=0.923-0.02x_1-0.0078x_2-0.028x_3+0.045x_1x_2-0.027x_1x_3-0.015x_2x_3-0.046x_1^2-0.028x_2^2-0.13x_3^2 \quad (4)$$

The predicted values of y by the above model are in close agreement with experimental data as evident from the last two columns of the Table 6.

Optimization of the equation (4) yielded the following values

Opt. Values of (scaled) independent variables: $x1 = -0.44222$; $x2= -0.47617$; $x3= -0.034131$

Opt. values of independent variables: $X_1 = 2.1156$; $X_2 = 1.2619$; $X_3 = 7.9317$

Opt.value of dependent variable; $Y_{opt} = 0.93063$

$R^2 = 0.88825$

Table 7
Regression coefficients estimation & their statistics

Coeff	Standard Error	t.stat	p Value
0.92386	0.027381	33.741	4.2997e-007
-0.020008	0.016767	-1.1933	0.28629
-0.0078208	0.016767	-0.46643	0.66052
-0.027987	0.016767	-1.6691	0.15596
0.04475	0.023713	1.8872	0.11779
-0.026786	0.023713	-1.1296	0.3099
-0.015335	0.023713	-0.64671	0.54633
-0.045682	0.024681	-1.8509	0.12341
-0.028443	0.024681	-1.1524	0.30125
-0.1295	0.024681	-5.2469	0.0033349

Regression ANOVA

Source	df	SS	MS	F	P
Regr	9.0000	0.0894	0.0099	4.4157	0.0583
Resid	5.0000	0.0112	0.0022		
Total	14.0000	0.1006			

Analysis of variance (ANOVA) for the response surface model Eq. (4) is summarised in Table 7. It is evident that equation is highly significant as is evident from the model F-value and a very low probability value (P model: 0.05). The goodness f the model can be checked by the R^2 statistic, which is 0.89, suggesting that 89% total variation for adsorbent removal is represented by the equation. The p-values of estimated coefficients indicate the significance of each coefficient, .the smaller the value of p, the more significant is the corresponding coefficient. Usually coefficients having p-values greater 0.05 are ignored from the regression equation. The negative values for the three Eigen values (-013, -0.06 & -0.01) obtained with coefficients matrix equation (Eigen values of matrix B of Eq. (2.8)) indicate that a maximum value of response is obtained which is also evident from the 3-D response plots (Fig 5, 6 & 7) in which the interactive effect of the two variables (with the third variable being fixed at its optimum value) on the biosorption of methylene blue is depicted.

Figure 5
Surface plot for MB adsorption with X_1& X_2

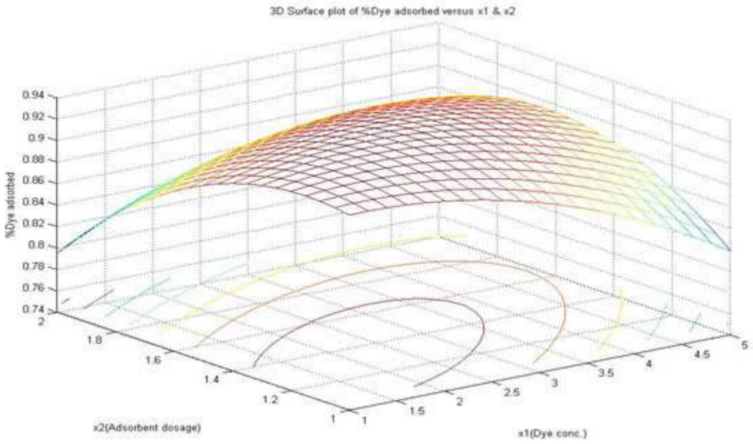

Fig 5 shows the response surface of methylene blue uptake when pH is fixed at its optimum value and the other two variables vary in the experimental domain. As evident from the 3-D figure, maximum dye uptake was obtained from the centre point of inner most contour (initial MB conc:2mg; ads dosage: 1.2g). Initial dye concentration in the range 1 to 3.5 with corresponding adsorbing dosage 1 to 1.4 favoured the maximum uptake of dye. At other concentrations of these two variables, methylene blue uptake decreased.

Figure 6
Surface plot for MB adsorption with X_1& X_3

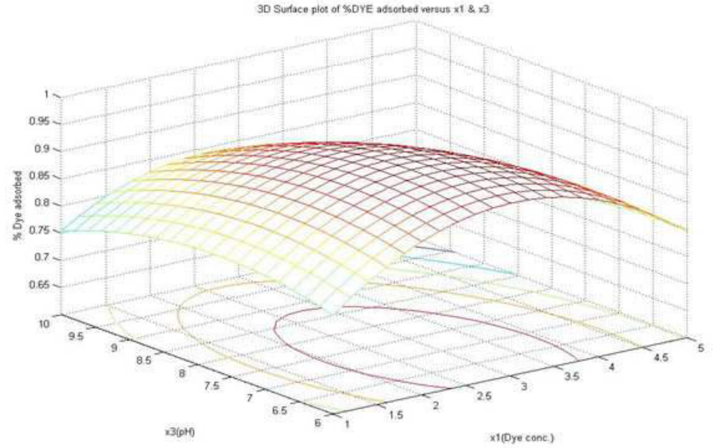

Fig.6 depicts 3-D response surface plot of methylene blue uptake with respect to the combined effect of initial MB conc. and pH when absorbent dosage is fixed at its optimum value. Initial MB conc. in the range (2 to4) and pH in the range (6 to 8.5) favoured maximum MB uptake.

Figure 7
Surface plot for MB adsorption with X_2& X_3

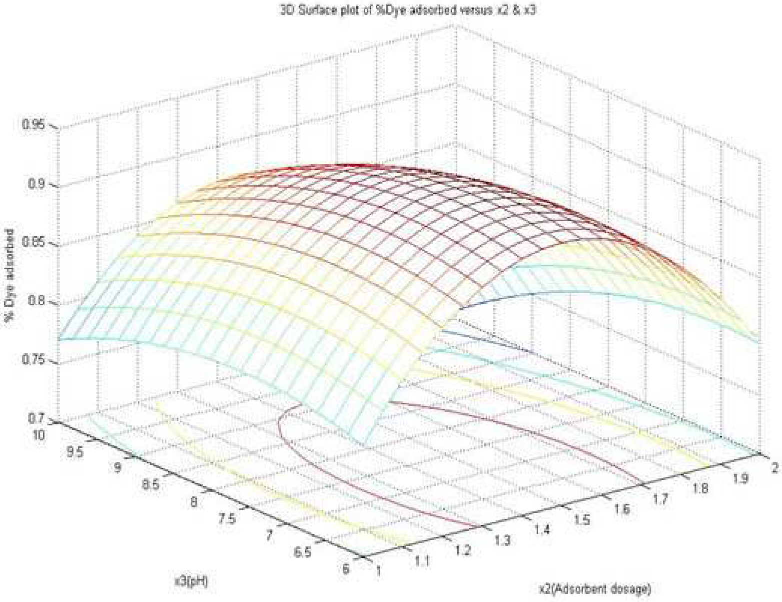

Fig 7 shows the combined effect of adsorbent dosage and pH on % MB uptake when the third variable is kept as its optimum. Adsorbent (*A.terrus*) dosage in the range 1.3 to 1.7 and the pH in the range of 6 to 9 favoured the maximum uptake of MB.

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