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# **Kinetic Study of Methylene Blue Removal on Immobilized Biomass Left after Enzyme Production Using Response Surface Methodology**

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## **Authors' contributions**

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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**Original Research Article**

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# **ABSTRACT**

**THE ROOM** 

**Aims:** Kinetic study of methylene blue dye removal, screening and optimization of different process parameters.

**Place and Duration of Study:** Department of Environmental Science and Engineering, Guru Jambheshwar University of Science and Technology, Hisar. June 2010 and July 2011.

**Methodology:** The spent biomass immobilized with Aspergillus flavus left after enzyme production dried at 40°C and dried biomass utilized for dye remo val. Biosorption kinetic experiments were carried out in 150 ml flasks by varying contact time from 5 to 180 min initial dye concentration and agitation rate was 200 mg/l and 100 rpm respectively at 30°C. Response surface methodology was applied for screening and optimization of process parameters for dye removal.

**Results:** Plackett-Burman design (PBD) and Box-Behnken design (BBD) was applied for screening of variables i.e. dye concentration, adsorbent dose, pH, temperature, contact time and agitation affecting dye decolorization. Variables were adsorbent dose (0.1- 0.5 g), dye concentration (100- 200 mg/L) and contact time (10- 60 min). Maximum decolorization 62.07% obtained at adsorbent

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dose 0.5 g and dye concentration of 100 mg/L after 35 min at pH 7 and temperature 30°C. Kinetic studies of dye adsorption showed that it followed pseudo-second order kinetics. **Conclusion:** The spent biomass immobilized with Aspergillus flavus left after enzyme production effectively removes methylene blue dye.

Keywords: Methylene blue; spent biomass; rice straw; dye; kinetics; RSM.

## **1. INTRODUCTION**

Many industries, such as paper, plastics, food, cosmetics, and textile, use dyes in order to color their products and also consume substantial volume of water. Among these industries, textile industry is considered as an intensive water consuming industry besides utilizing a wide variety of chemicals and dyes [1-2].

The presence of dyes in effluents is a major concern due to their adverse effects to many forms of life. The discharge of dyes in the environment is a matter of concern for both toxicological and esthetical reasons [3-4]. Industries generate a considerable amount of colored wastewater $3$ ; this interferes with its intended beneficial use. It is estimated that more than 100,000 commercially available dyes with over  $7 \times 10^5$  tons of dyestuff are produced annually [5-7]. It is recognized that public perception of water quality is greatly influenced by color. The color is first contaminant to be recognized in wastewater. The presence of even very small quantity of dyes in water less than 1 mg/L for some dyes is highly visible and undesirable [8-9].

Methylene blue (MB) is the most commonly used substance for dying cotton, wood and silk. It can cause eye burns which may be responsible for permanent injury to the eyes of human and animals. On inhalation, it can give rise to short periods of rapid or difficult breathing while ingestion through the mouth produces a burning sensation and may cause nausea, vomiting, profuse sweating, mental confusion and methemoglobinemia [10-11]. Therefore, the treatment of effluent containing such dye is of interest due to its harmful impacts on receiving water [12].

During the past few decades, several physical, chemical and biological decolorization methods have been reported; however, few have been accepted by the paper and textile industries [13-14]. Amongst the numerous techniques of dye removal, adsorption is reported to give the best results as it can be used to remove different types of coloring materials [14-16]. Recently, numerous approaches have been studied for the development of cheaper and effective adsorbents. Many non-conventional low-cost adsorbents, including natural materials, biosorbents and waste materials from agriculture industry have been proposed by several workers [17-20]. In the present study, fungus immobilized rice straw left after enzyme production [21] has been used as adsorbent for dye removal.

#### **2. MATERIALS AND METHODS**

#### **2.1 Biosorbent Preparation**

Fungus immobilized microwave alkali pretreated rice straw was used for dye decolorization. Aspergillus flavus grown on microwave alkali pretreated rice straw under solid state fermentation for lignocellulolytic enzyme production [22] was used as adsorbent. The spent immobilized biomass left after enzyme production dried at 40°C and dried biomass utilized for dye decolorization.

## **2.2 Preparation of Dye**

Stock solution of MB was prepared by dissolving 1.0 g of each dye in 1000 mL of double distilled water. Working solutions of different concentrations (5–500 mg/L) were prepared by further dilutions. Standard curves were developed through the measurement of absorbance of dye solutions by UV/Visible Spectrophotometer (T80 UV/VIS Spectrophotometer, PG Instruments Ltd.).

## **2.3 Biosorption Kinetics**

Biosorption kinetic experiments were carried out in 150 ml flasks by varying contact time from 5 to 180 min initial dye concentration and agitation rate was 200 mg/l and 100 rpm respectively at 30°C. 1.5 ml sample was taken after 5 min interval and centrifuged at 10,000 rpm in triplicates and analysed spectrophotometrically at λmax 673 nm. The percentage of dye removal and adsorption of dye at equilibrium time on spent immobilized biomass of rice straw was calculated using the following equation [23]:

MB decolorization (Percentage) = 
$$
(C_0 - C_e) \div C_0 \times 100
$$
 (1)

Adsorption capacity 
$$
\left(\frac{mg}{g}\right) q_e = \left(C_0 - C_e\right) \times V \div M
$$
 (2)

Where  $C_0$  is the initial dye concentration (mg/L) and Ce is the residual concentration of the dye (mg/L) at different time intervals, qe is the quantity of MB adsorbed on adsorbent at the time of equilibrium (mg/g), V is the volume (L) of solution and M is the mass of adsorbent (g) taken for experiment.

## **2.4 Optimization Using RSM**

Response surface methodology was developed by Box and collaborators in the fifties [24]. This term was originated from the graphical perspective generated after fitness of the mathematical model and has been widely adopted in texts on chemo metrics. RSM consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design [24].

#### **2.4.1 PBD study**

The Plackett-Burman experimental design, a fractional factorial design, was used in this work to identify which variable(s) has a significant effect on percentage uptake of dye. Six independent variables (initial dye concentration, adsorbent dose, pH, temperature, contact time and agitation rate) (Table 2) in twelve combinations were organized according to the Plackett-Burman design matrix. For each variable, a high (+1) and low (-1) level was tested (Table 2). All trials were performed in triplicate. Experimental design and statistical analysis of data were done by for parameters screening using Design Expert Version 8.1.1 (trial version).

#### **2.4.2 BBD study**

The experiments were based on a Box-Behnken design with a quadratic model in order to study the combined effects of three independent variables (contact time, adsorbent dose and dye concentration) screened out from PBD study and each variable varied at three levels for decolorization and conducted experiments as shown in Table 4. The value of the dependent response was the mean of three replications. Contact time (5-180 min.), adsorbent dose (0.1– 0.5 g) and dye concentration (100–200 mg/L) were independent variables and methylene blue removal was the response (dependent) variable. Experiments were performed at pH 7 and temperature 30°C already optimized [25]. The statistical analysis of the data was performed using "Design Expert" software (Trial version 8.1.1, Stat-Ease, Inc., Minneapolis, USA).

# **3. RESULTS AND DISCUSSION**

#### **3.1 Biosorption Kinetic**

Biosorption kinetics was studied for adsorption of MB of initial concentration 200 mg/L. The rate of sorption by spent immobilized biomass of rice straw left after enzyme production decreased with increase of time, and after 60 min equilibrium was achieved (Fig. 1). Various kinds of kinetic models have been suggested to understand the solute sorption mechanism from aqueous solution on to any adsorbent [23,25]. In order to find out the adsorption mechanism, constants of adsorption were determined using pseudo-first order equation of Lagergren and pseudo-second order equation [26-28].

$$
\frac{d q}{dt} = k_1 (q_e - q_t)^2 \tag{3}
$$

Where,  $k_1$  (min<sup>-1</sup>) is the Lagergren rate constant, which was determined using pseudo-first order equation of Lagergren (3) and qe (mg/g) and qt (mg/g) are the amounts of dye adsorbed per unit adsorbent at equilibrium and at time t, respectively. For boundary layer conditions i.e. initial time  $(t = 0)$ ,  $qt = 0$  and at any time  $(t > 0)$ , integrated form of Eq. (3) is:

$$
\log (q_e - q_t) = \log(q_e) - \left(\frac{k_1}{2.303}\right)t \tag{4}
$$

Linear form of the pseudo-second order model, Ho and MacKay's pseudo-second order model is shown as Eq. (5):

$$
\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right)t\tag{5}
$$

Where qe is amount of dye adsorbed at equilibrium (mg/g) and  $k_2$  is the equilibrium rate constant of pseudo-second order (g/mg min). The linear graph between the t/ $q_t$  and contact time (t) provides the intercept  $(1/\kappa_2 q_e^2)$  and the slopes ( $1/q_e$ ) from which the values of  $k_2$  and  $q_e$ 

were calculated. Values of  $q_{e}^{cal}$  and  $q_{e}^{exp}$  were calculated and found very close to each other in pseudo second order kinetics (Table 1). The value of regression correlation coefficient  $(R^2)$ was 0.997 very close to unity which proves that MB adsorption using spent immobilized biomass left after ethanol production follows pseudosecond order kinetic model.

## **3.2 Statistical Optimization Study**

#### **3.2.1 PBD study**

The effects of the factors screened by PBD were estimated by statistical analysis. Table 3 shows effect of the standardized effects and contribution percentages of the variables tested in PBD. It was found from Table 3 that adsorbent dose, dye concentration, contact time were significant variables, whose contribution percentage was near to 94% on dye removal. Three factors dye concentration; adsorbent dose and contact time were selected for further study by RSM. Agitation rate was fixed at 100 rpm. pH and temperature was set 7 and 30°C already optimized [25].

#### **3.2.2 BBD study**

On basis of results of PBD, experiment was designed for BBD to discover optimum conditions. Three significant factors identified from PBD i.e. dye concentration, adsorbent dose and contact time were selected as major variables for BBD study and each variable varied at three levels for dye removal. The value of the dependent response was the mean of three replications. Dye concentration (100-200 mg/L), adsorbent dose (0.1–0.5 g) and contact time (10- 60 min) were considered as the independent variables while removal of MB was the response (dependent) variable. Study of respective model equation was examined by using analysis of variance (ANOVA) and F-test as shown in Table 5. The Model F-value of 28.9 implies the model is significant. Lack of fit analysis, which measures the failure of a model to represent the data in the experimental domain, was carried out. The fitness of model was also indicated by the coefficient of determination,  $R^2$ , and Adj.  $R^2$ which were found to be 0.97 and 0.94, respectively. The p- value i.e. probability factor for the model was less than 0.05 which indicated that the model was significant. In this study, p-value of A, B, C, AB, AC and BC were significant as the values were less than 0.05 as shown in Table 5.

$$
Y_1 = b_0 + b_1A + b_2B + b_3C + b_{11}A^2 + b_{22}B^2
$$
  
+  $b_{33}C^2 + b_{12}AB + b_{13}AC$   
+  $b_{23}BC$  (6)

% Removal =  $+17.17 + 17.23 * A - 10.72 * B$  $+9.82 * C - 9.88 * A * B + 9.05$  $* A * C - 9.89 * B * C$  $(7)$ 

where  $b_0$  is the intercept,  $b_1$ ,  $b_2$ ,  $b_3$ , linear coefficients;  $b_{11}$ ,  $b_{22}$ ,  $b_{33}$ , squared coefficients;  $b_{12}$ ,  $b_{13}$ ,  $b_{23}$ , interaction coefficients; A, B, C represent coded levels of the independent variables.



**Fig. 1. Biosorption kinetics of MB dye on immobilized biomass of rice straw left after enzyme production at pH 7.0, temperature 30°C, adsorbent d ose (1 g/100 ml) and initial dye concentration 200 mg/L** 

Dye concentration (mg/L)	$\mathsf{a}^{\overline{\mathsf{exp}}}$ e (mg/g)	<b>Pseudo-first order</b>			<b>Pseudo-second order</b>		
$200$ ppm	128.8	$\overline{a}$ <sup>cal</sup> е (mg/g)	M1 $(min^{-1}$		$\sqrt{a}$ е (mg/g)	$K_2$ (q/mq/min)	D'
		0.77	0.28327	0.98	31.2	0.011	0.99

**Table 1. Kinetic parameters for MB adsorption on spent biomass left after enzyme production** 



Run No.	<b>Dye</b> concentration (mg/l)	<b>Adsorbent</b> dose(g)	рH	Temp $(\mathfrak{C})$	contact time (min.)	<b>Agitation</b>	percentage removal
	100	0.1	$\overline{2}$	40	180	Stationary	27.50
2	200	0.1	$\overline{2}$	20	180	Stationary	29.47
3	200	0.5	2	40	180	100	29.26
4	200	0.5	9	20	180	Stationary	30.19
5	100	0.1	$\mathcal{P}$	20	5	100	74.68
6	100	0.5	2	20	5	stationary	47.94
	200	0.5	$\mathcal{P}$	40	5	100	29.33
8	200	0.1	9	40	5	stationary	42.18
9	100	0.5	9	20	180	100	80.16
10	100	0.5	9	40	5	stationary	88.23
11	200	0.1	9	20	5	100	62.57
$12 \overline{ }$	100	0.1	9	40	180	100	28.653

**Table 3. Levels and results of variables tested in PBD** 



Fig. 2 (a) shows the interaction between contact time and dye concentration. It was observed that the rate of removal of MB dye increases with an increase in contact time up to a certain extent. Further increase in contact time does not increase the uptake due to the deposition of dyes on the available adsorption site on adsorbent material [27]. Maximum decolorization i.e. 43.5% was obtained between 100-125 mg/L of dye concentration and 47.50-60 min. of contact time. Fig. 2 (b) shows interaction between contact time and adsorbent dose. As shown in Fig. 2 (b), the percentage of decolorization increased as the adsorbent dose increased. The higher percentage removal at high biosorbent dosage may be attributed to the presence of large surface area of biosorbent for biosorption [29]. Maximum decolorization i.e. 45.68% was obtained between 0.40-0.50 g of adsorbent dose. Fig. 2 (c) shows interaction between dye

concentration and adsorbent dose. In the biosorption mechanism, at the beginning the dye molecules were adsorbed externally and the biosorption rate increased rapidly. When the external surface became saturated, the dye molecules were adsorbed into the porous structure of biomass [30-32]. The adsorption process is highly dependent on initial concentration of dye in solution and percentage removal of MB decreased as its initial concentration increased [33-35]. The increase in percentage decolorization, at higher adsorbent dose is due to a very fast superficial adsorption onto the adsorbent surface that produces a lower solute concentration in the solution. However, dye uptake increases with the increase in initial dye concentration for constant adsorbent dose. This may be due to the presence of more number of dye molecules in the solution [36-37].



**(c)** 

**Fig. 2. Response surface plots shows interactive effects (a) between contact time (min.) and dye concentration (mg/l). (b) between contact time (min.) and adsorbent dose (g). (c) between dye concentration (mg/l) and adsorbent dose (g)** 





<b>Source</b>	Sum of	DF	Mean	<b>F</b> value	Prob > F	
	squares		square			
Model	5317.99	9	590.89	28.95	0.0001	Significant
A	2373.95		2373.95	116.31	< 0.0001	
в	918.92		918.92	45.02	0.0003	
C	772.05		772.05	37.83	0.0005	
A <sub>2</sub>	51.67	1	51.67	2.53	0.1556	
<b>B2</b>	3.34		3.34	0.16	0.6979	
C <sub>2</sub>	96.28		96.28	4.71	0.0664	
AB	390.66		390.66	19.14	0.0033	
AC	327.61		327.61	16.05	0.0051	
ВC	391.05		391.05	19.15	0.0032	
Residual	142.87		20.41			
Lack of fit	80.43	3	26.80	1.71	0.3007	Not significant

**Table 5. ANOVA for selected Box–Behnken design for dye removal** 

\*Prob > F value less than 0.05 indicates that model terms were significant and more than 0.05 were insignificant. Model F-Value: 28.9; R-Squared: 0.97; Adj R-Squared: 0.94

# **4. CONCLUSION**

Waste left after enzyme production was found a good adsorbent for removal of methylene blue dye. After pbd study, it was found that 0.5 g and 100 mg/l were optimum adsorbent dose and dye concentration respectively after 35 min. Maximum decolorization was 62.07% obtained at pH 7 and temperature 30°C for dye removal. It was found that pseudo-second order kinetic was better fitted than pseudo-first order kinetics. So it was a cheap and effective adsorbent for removal of methylene blue.

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# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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Manju et al.; JALSI, 6(3): 1-9, 2016; Article no.JALSI.27723

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