Pallavi.P et al /J. Pharm. Sci. & Res. Vol. 9(12), 2017, 2319-2328

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Experimental and Statistical Modeling on the Biosorption of Methylene blue from an Aqueous Solution using Raphanus raphanistrum Leaves

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Abstract

Experiments were conducted with the process parameters such as contact time, initial dye concentration, solution pH, biosorbent dosage, biosorbent particle size and temperature for the feasibility of Raphanus Raphanistrum biosorbent for the removal of Methylene blue dye. Equilibrium isotherms were analyzed using the Langmuir, Freundlich, Temkin and DR isotherm models and the Langmuir isotherm was fitted well. The kinetic studies for methylene blue biosorption showed rapid sorption dynamics with a pseudo second order kinetic model. A thermodynamic parameters such as enthalpy(ΔH^0), entropy (ΔS^0) and Gibbs free energy (ΔG^0) were evaluated which showed that methylene blue biosorption onto Raphanus Raphanistrum was exothermic and spontaneous process. The effect of imperative parameters like contact time, solution pH, initial dye concentration and temperature were processed under optimized conditions by using response surface methodology (RSM) taking into account central composite design(CCD).

Keywords: Methylene blue, Raphanus Raphanistrum Leaves (RR), biosorption isotherms, Kinetic studies, thermodynamic studies, Central Composite Design (CCD).

1. Introduction

Environmental compliance requirements have become increasingly difficult to attain in both wastewater discharge and chemical handling. Biosorption is relevant in environmental pollution and protection with reference to water and wastewater treatment [1]. Biosorption by solids decreases the toxicity of the wastewater or removes non-safe organic materials from industrial effluents [2,3]. Studies carried through environmental biotechnology have shown that many biosorbents present in the nature have great capacity for removal of dyes [4]. Decolourization of dye-containing effluent is becoming an obligation both environmentally and for water re-use. Over $7x10^5$ tons of these dyes are produced annually world-wide. It is estimated that 10-15% of these chemical compounds are discharged into waste streams by the textile industry. Dyes and pigments represent one of the problematic groups; they are emitted into wastewaters from various industrial branches, mainly from the dye manufacturing and textile finishing. Thus, the toxicity, bio-accumulation and persistence of these dyes are transmitted through the food chain and the environment to cause environmental and human health problems. Dyes can be classified as anionic (direct, acid, and reactive dyes), cationic (basic dyes) and non-ionic (disperse dyes) [5]. Methylene blue has wider applications, which include coloring paper, temporary hair colorant, dyeing cottons, wools, coating for paper stock, etc. In addition, dyes are toxic to some organisms and hence harmful to aquatic animals. Acute exposure to methylene blue will cause increased heart rate, vomiting, shock, Heinz body formation, cyanosis, jaundice, quadriplegia and tissue necrosis in humans. Furthermore, the expanded uses of azo dyes have shown that some of them and their reaction products such as aromatic amines are highly carcigenic [6,7]. However, a recent study conducted under the National Biodiversity strategy and Action Plan (BSAP) has revealed that chemical colors have all been wiped out India's wonderful vegetable dyes.

The most commonly used method for color removal is biological oxidation and chemical precipitation. Several research works had been performed to search the efficiency and low-cost materials to remove methylene blue and other basic dyes from an aqueous solution, including rice husk [8], beech sawdust [9], agro-industry wastes [10] and activated carbon from data pits [11]. Hence, the conventional methods used in sewage treatment, such as primary and secondary treatment systems, are unsuitable [12]. Currently biosorption process is proved to be one of the effective and attractive processes for the treatment of these dye-bearing wastewaters [13-15]. Biosorption is attributed to its low cost, easy availability, simplicity of design, high efficiency, ease of operation, biodegradability and ability to treat dyes in more concentrated forms [16].

Hence, from the past research many low cost waste materials were used as the alternative biosorbents for the removal of methylene blue (MB) from an aqueous solution. The leaf powder of Raphanus Raphanistrum has high surface area when compared to the other biosorbents. By maintaining the proper conditions of pH, contact time etc, the properties like surface area and pore volume of the leaf

powder has been developed, proceeded in effective biosorption behavior. The present study deals with the removal of Methylene blue dye by the use of Raphanus Raphanistrum leaves from an aqueous solution.
Research studies commonly used different techniques for process Optimization. RSM is one of the relevant multivariate techniques which can deal with multi-variant experimental design strategy, statistical modeling and process optimization [17]. The relation between process parameters and a set of experimental parameters or variables are examined. The present work stands to assess the effects of process parameters like contact time, solution pH, initial dye concentration and temperature to identify the optimum conditions using a central composite design (CCD).

2. Materials And Methods

***Preparation of powder from* Raphanus Raphanistrum Leaves**

Raphanus Raphanistrum leaves were collected from Vignan's Engineering College, Vadlamudi, AP. The leaves were segregated, washed several times with deionized water until the wash water contains no dirt. The washed leaves were completely dried under sunlight for 30 days and powdered using domestic mixer. The dried powder was sieved for 63-212 µm.

Preparation of stock solution

Biosorbate solution was prepared from Methylene Blue dye. 0.1 g of dye was weighed and a standard stock solution of concentration 1000 mg/L was prepared in double distilled water and further working solutions of concentrations (25, 50, 75 and 100 mg/L) were prepared and stored in the stoppered bottles, used as required.

3. Experimentation

The biosorption studies were conducted under effective process parameters of solution pH 2-10, dye concentrations 25-100 mgL^{-1}, weight of the biosorbent 0.02-0.2 g and particle size of the biosorbent vary from 63 (242 mesh) -212 (72 mesh) µm. Agitation speed of 180 RPM was kept steady in the orbital shaker with the suitable time intervals from 5-135 min. The mixed biosorbent solutions were taken out, filtered by using filter papers and analyzed for Methylene blue dye concentration in an UV Spectrophotometer at 668nm.
The % biosorption is given as

$$\% \, Biosorption = (C_i - C_f)/C_i \, x100 \qquad (1)$$

The dye uptake onto RR biosorbent is conducted as

$$q_t = \frac{V(C_i - C_f)}{1000w} \qquad (2)$$

Where q_t is the amount of dye adsorbed on the RRL biosorbent surface *(mg/g),*
C_i is the initial concentration of solute in the solution before biosorption *(mg/L),*
C_f is the final concentration of solute in the solution after biosorption *(mg/L)*,
V is the volume of the dye solution *(ml)* and
w is the weight of the biosorbent (g).

4. Results And Discussion

Effect of Contact time:

For different initial dye concentrations from 25 mg/L - 100 mg/L, the biosorption efficiency with respect to contact time is represented in Fig.1. As the contact time increased the percentage biosorption gradually increased and reaches an equilibrium value at 75 min, where maximum amount of percentage biosorption was obtained. At the initial stages of contact time more active sites are present for biosorption. The %biosorption was constant even after increase in contact time from 75 min. The biosorption efficiency was increased from 84.52% to 98.8% with a contact time of 75 min at 25 mg/L initial dye concentration.

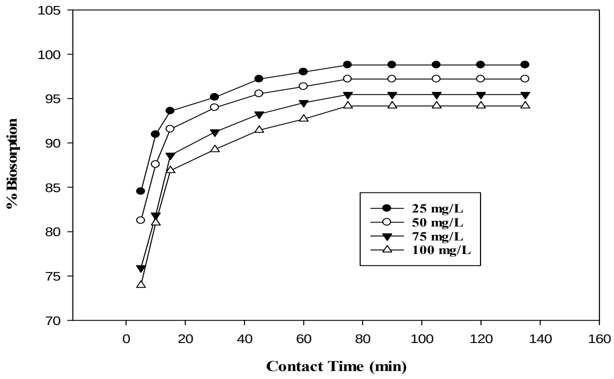

Fig.1: Effect of contact time on the biosorption efficiency of MB dye on RR biosorbent

Effect of solution pH:

The solution pH highly influences the biosorption capacity. pH majorly affects the surface charge of biosorbents as well as degree of ionization of dyes in the aqueous solution. Fig-2 shows the effect of pH on biosorption of MB dye at a contact time of 75 min and a temperature of 303 K. In highly acidic medium, the surface of RR biosorbent may become negatively charged, which enhances the positively charged methylene blue cations through electrostatic forces of attraction [18]. The increase in pH leads to formation of precipitate that shifts the reaction kinetics and equilibrium characteristics. The percentage biosorption is low initially, increases gradually and attains equilibrium at pH 6. At pH 6 maximum percentage biosorption efficiency of 98.8% was attained.

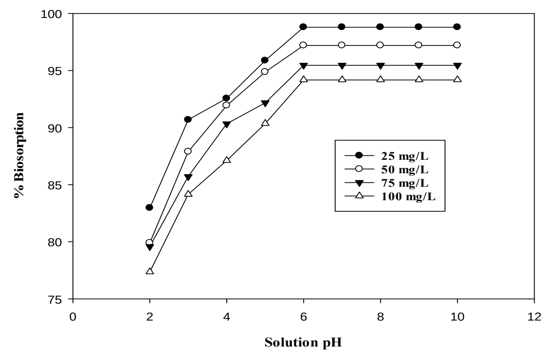

Fig.2: Effect of pH on the biosorption efficiency of MB dye on RR biosorbent

Effect of *Initial dye concentration:*

For effective biosorption, the initial concentration of the biosorbate influences the rate of biosorption. The equilibrium biosorption capacity of the biosorbent decreased with an increase in initial dye concentration, as shown in Fig.3. Dye removal is highly concentration dependent. The decrease of biosorption capacity with an increase of dye uptake is probably due to high driving force for mass transfer. At 303 K, when initial dye concentration increases from 25 mg/L to 100 mg/L, the dye uptake increased from 12.35 to 47.09 mg of methylene blue per gram of RR biosorbent.

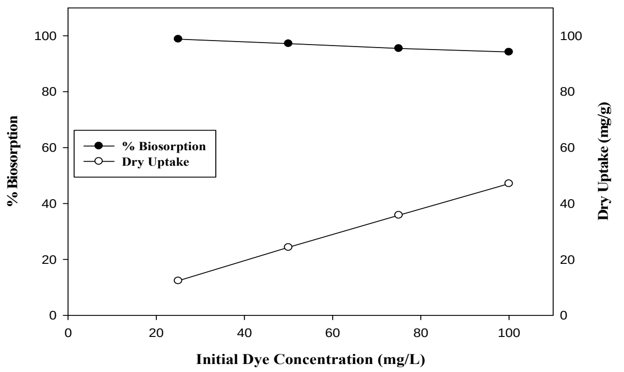

Fig.3: Effect of initial dye concentration on the biosorption efficiency of MB dye on RR biosorbent

Effect of Biosorbent Dosage:

As the weight of the biosorbent dosage increases there is a proportional increase in the percentage of biosorption (Fig.4). The percentage biosorption increased with an increase in biosorbent dosage from 0.02 to 0.1g and remained constant even after increasing the dosage from 0.1g. The increase in biosorption with the increasing dosage of biosorbent is basically due to biosorption sites remained unsaturated during the biosorption reaction [19]. The percentage biosorption increased from 82.96 to 98.8%.

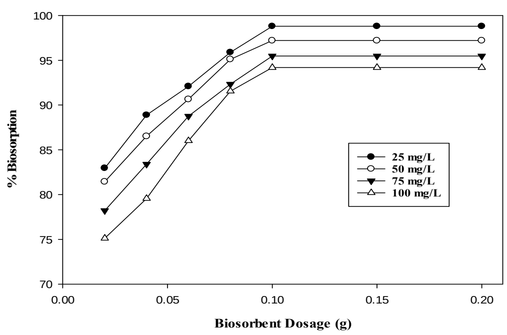

Fig.4: Effect of biosorbent dosage on the biosorption efficiency of MB dye on RR biosorbent

Effect of average biosorbent size on RR biosorbent

The biosorption efficiency is influenced by the number of active sites per unit volume of biosorbent for solid-liquid interface. The percentage biosorption decreased from 98.8% to 79.6% with an increase of particle size from 63µm to 212 µm (fig.5). When compared with larger particles, the smaller biosorbent particles will have more surface area and will have more contact with the dye solution, where rapid biosorption process takes place with high masstransfer.

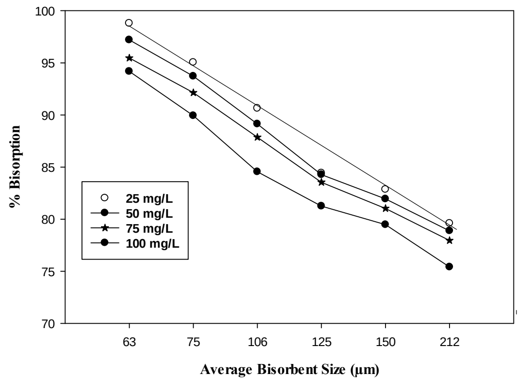

Fig.5: Effect of average biosorbent size on the biosorption efficiency of MB dye on RR biosorbent

Effect of temperature on RR biosorbent

The effect of temperature on biosorption of MB dye is shown in fig.6. The percentage biosorption decreased as the temperature increased from 303K to 343K for all initial dye concentrations. At high temperatures, the thickness of the boundary layer decreases, due to the increased tendency of the dye to escape from the biomass surface to the solution phase, which results in a decrease in biosorption as temperature increases [20]. The decrease in biosorption with increasing temperature suggests weak adsorption interaction between biomass surface and the dye [21]. The maximum biosorption was observed at 98.8% at 303K at a contact time of 75 min.

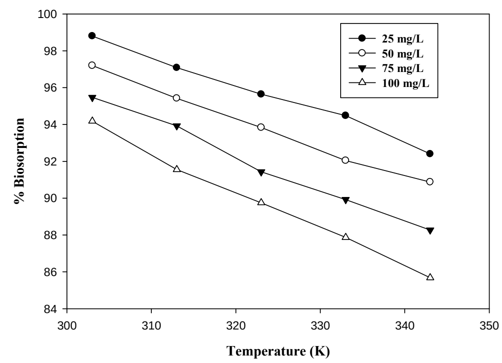

Fig.6: Effect of temperature on the biosorption efficiency of MB dye on RR biosorbent

Suitability of Isotherms to fit the Data

The Freundlich isotherm is derived by assuming heterogeneous surface with a non-uniform distribution of heat over the surface. The linear form of the Frendluich isotherm model is given by

$$\ln q_e = \ln K_f + \frac{1}{n_f} \ln C_e \qquad (3)$$

From the Freundlich isotherm model, q_e and C_e are the equilibrium dye uptake (mg/g) and concentration (mg/L) respectively. A graph was drawn between $\ln C_e$ vs. $\ln q_e$. The values of K_f and $1/n_f$ are the freundlich constants evaluated by using equation (3). As the value of percentage biosorption decreases with increasing temperature the value of $1/n_f$ is decreasing simultaneously, which represents the biosorbent sites are blocked up by the dye solution. The high value of R^2 represents the fitness of the Freundlich isotherm. The values of constants are given in Table-1.

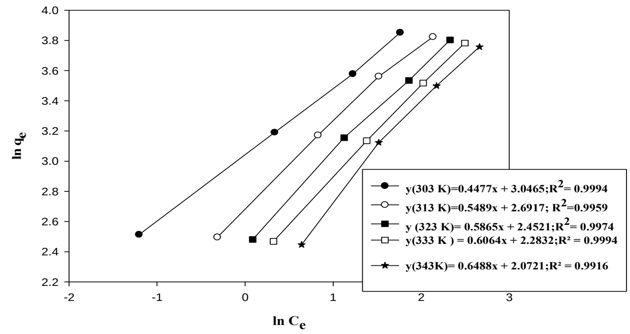

Fig.7: Freundlich isotherms at different temperatures for biosorption of MB dye onto RR biosorbent

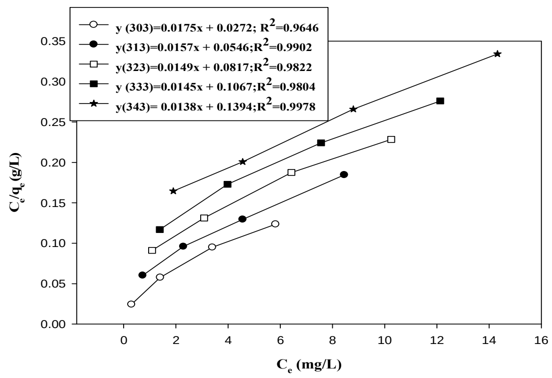

Fig.8: Langmuir isotherms at different temperatures for the Biosorption of MB dye onto RR biosorbent

The linearized Langmuir equation was applied to analyze the equilibrium experimental data. The equation is given as:

$$\frac{1}{q_e} = \frac{1}{q_{max} K_L C_e} + \frac{1}{q_{max}} \qquad (4)$$

C_e and q_e are the concentration (mg/L) and dye uptake (mg/g) at equilibrium respectively. A plot of C_e vs. C_e/q_e was plotted for different initial dye concentrations. The value of constants K_L and q_{max} were calculated from equation (4) and represented in Table-1. The value of biosorption capacity (q_{max}) was found to be increasing with an increase in temperature, which represents variations in surface charges present in the biosorbents.

The separation factor (R_L) was evaluated by using the equation (5), where C_i is the initial concentration (mg/L). The values of R_L are between 0 to 1, which represents the biosorbent is favourable with an increase in the initial dye concentration.

$$R_L=1/(1+K_L C_i) \qquad (5)$$

Temkin isotherm gives an idea about biosorption potential and to assess the biosorption energy variations during biosorption process. Temkin equation is represented as

$$q_e = B_T \ln(A_T) + B_T \ln(C_e) \qquad (6)$$

where q_e and C_e are the dye uptake (mg/g) and concentration (mg/L) at equilibrium. B_T and A_T are the linear temkin isotherm constants which are determined from equation (6). A graph of ln C_e vs. q_e was drawn and the values are tabulated at different temperatures in Table-1. The correlation coefficient R^2 value increased with an increase in temperature. However, the values of temkin correlation coeffiecient are less when compared with Freundlich isotherm, which shows that Freundlich isotherm was best fitted with data than temkin isotherm.

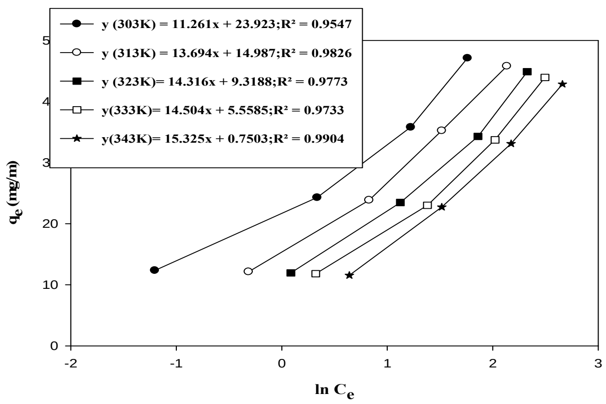

Fig.9: Temkin isotherms at different temperatures for biosorption of MB dye onto RR Biosorbent

Table: 1. Isotherms constants for MB dye biosorption onto RR biosorbent at C_i=25-100 *mg/L*, *w* = 0.1g, d= 63 *µm*, T= 303 K, pH= 6 and t = 75*min*.

Isotherm Model	Parameters	Different Temperature (K)				
		303	313	323	333	343
Freundlich	K_f *(mg/g)/(L/g)*^{n}	21.0415	14.7567	11.6127	9.8080	7.9414
	n_f *(L/g)*	2.2336	1.8218	1.7050	1.6490	1.5413
	R^2	0.9994	0.9959	0.9974	0.9994	0.9916
Langmuir	q_{max} *(mg/g)*	57.1428	63.6942	67.1140	68.9655	72.4637
	K_L *(L/g)*	0.6433	0.2875	0.1823	0.1358	0.0989
	R^2	0.9646	0.9902	0.9822	0.9804	0.9978
	$R_L=1/(1+K_L C_o)$	0.0585	0.1221	0.1799	0.2275	0.2879
Temkin	A_T *(L/g)*	8.3679	2.9874	1.9173	1.4670	1.0501
	B_T*(L/mg)*	223.70	190.03	187.58	190.88	186.08
	R^2	0.9547	0.9826	0.9773	0.9733	0.9904

Kinetic Modeling

The pseudo-first-order, pseudo-second-order and the Elovich kinetic models were used to test the controlling mechanism of biosorption process such as mass transfer and chemical reaction. The biosorption equilibrium rate is much faster at the biosorbent site, due to the presence of dye molecules present in the industrial wastewater. The commonly used pseudo-first-order model generally expressed as

$$\ln(q_e - q_t) = -k_f t + \ln q_e \qquad (7)$$

Where q_t (mg/g) is the weight of MB dye adsorbed on biosorbent surface at time *t,* and k_f (min^{-1}) is the first-order biosorption process rate constant. A plot of t verses *ln* $(q_e - q_t)$ at different concentrations (25 – 100 mg/L) using the dye at optimum conditions of pH 6 and temperature 303K was plotted in Pseudo-first order kinetics. Fig.11 shows the linear plot of pseudo-first-order kinetic model, the values are shown in Table-2.

Second-order kinetic model is expressed as

$$\frac{t}{q_t} = \frac{1}{q_e} t + \frac{1}{k_s q_e^{\,2}} \qquad (8)$$

where k_s (g mg^{-1} min^{-1}) is the second-order biosorption process rate constant. A linear relationship is developed for the applicability of the second-order kinetic model by plotting t vs. t/q as shown in Fig.12. The constants are calculated from equation (8). The increase of correlation coefficient indicates that both physisorption and chemisorptions takes place. The rate limiting step is the chemisorption that maintains the overall biosorption process. The pseudo second order was fitted with the data well.

The Elovich model gives the information about the type of biosorption mechanism (Physical or Chemical). It is expressed as

$$q = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln t \qquad (9)$$

Where α is initial rate and β representing activation energy (g/mg). A straight line plot of *lnt* verses q was drawn where the constants were obtained from the slope and intercept shown in Fig.13 and calculated from equation (9).The correlation coefficients are summarized in Table-2, indicate that for higher concentrations the Elovich model was not suitable to fit the data.

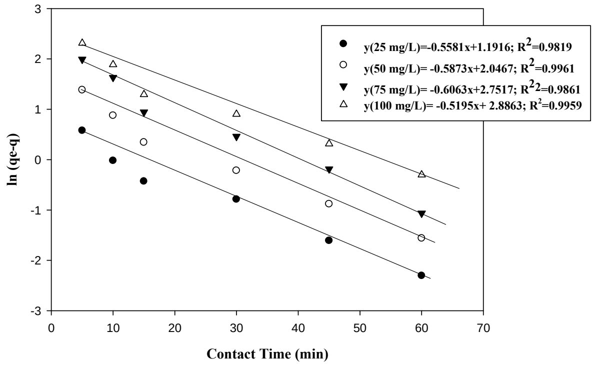

Fig.11: Pseudo First order Kinetic model for biosorption of MB dye onto RR biosorbent.

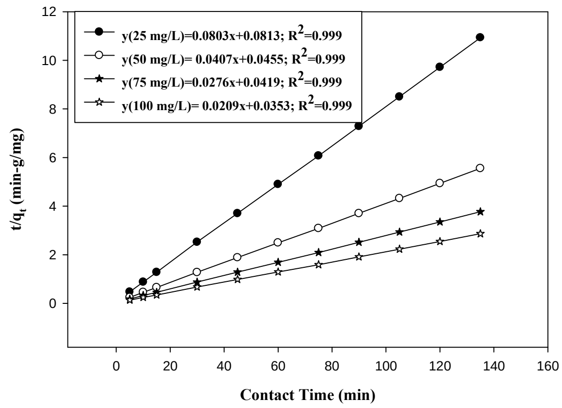

Fig: 12. Second order Kinetic model for biosorption of MB dye onto RR biosorbent.

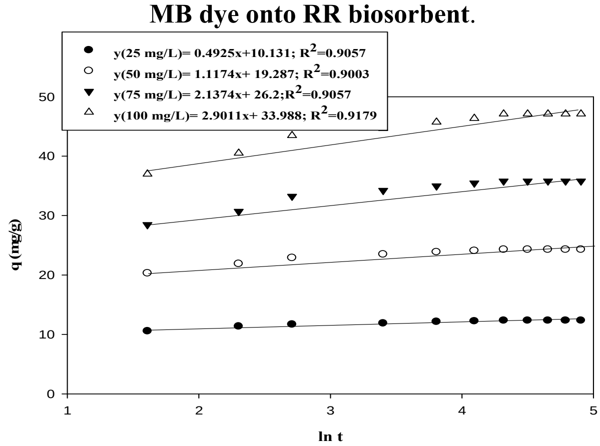

Fig: 13. Elovich kinetic model for the biosorption of MB dye onto RR biosorbent.

Table.2: Kinetic rate constants for MB dye biosorption onto RR at C_i = 25-100 mg/L, *w*= 0.1g, *d* =63-212 µm, *T*= 303 K, *pH*= 6 and *t* = 75 min.

C_i (mg/L)	Pseudo-first-order			Pseudo-Second-order			Elovich Model		
	k_f (min^{-1})	q_e (mg/g)	R^2	k_s (g/mg.min)	q_e (mg/g)	R^2	β (g/mg)	α (mg/g.min)	R^2
25	1.285	3.29	0.9819	0.079	12.453	0.999	2.0304	$422.52*10^6$	0.9057
50	1.352	7.74	0.9961	0.036	24.570	0.999	0.8949	$35*10^6$	0.9003
75	1.396	15.66	0.9861	0.018	36.231	0.999	0.4678	$449.58*10^3$	0.9057
100	1.196	17.92	0.9959	0.012	47.846	0.999	0.3446	$355.19*10^3$	0.9179

Table.3.Thermodynamic parameters for the biosorption of MB dye using RR biosorbent

C_i (mg/L)	$-\Delta H^o$ (kJ/mol)	$-\Delta S^o$ (kJ/mol.K)	$-\Delta G^o$(kJ/mol)				
			303 K	313 K	323 K	333 K	343 K
25	39.11	0.09	11.84	10.94	10.04	9.14	8.24
50	26.83	0.06	8.65	8.05	7.45	6.85	6.25
75	22.63	0.05	7.48	6.98	6.48	5.98	5.48
100	20.77	0.05	5.62	5.12	4.62	4.12	3.62

Thermodynamic studies

The Van't Hoff equation represents the thermodynamic parameters as

$$\ln K_D = \Delta S^o/R - \Delta H^o/RT \quad (10)$$

Where ΔH^o *is standard enthalpy*, ΔS^o *is standard entropy* and ΔG^o is standard Gibbs free energy, R is the universal gas constant (8.314 J/mol K), T is the absolute temperature (K). The thermodynamic parameters give an evidence of direction to the biosorption process. ΔH^o *and* ΔS^o are calculated from Equation (10).

The relationship between ΔG^o and K_D is given by the following equation,

$$\Delta G^o = -RT \ln K_D \quad (11)$$

The Van't Hoff plot was plotted between 1/T vs. ln K_D, shown in fig.10 for different concentrations of the dye. From equation of ΔG^o, ΔH^o and ΔS^o represents the reaction is exothermic. At different initial dye concentrations, the feasibility of percentage of biosorption decreased at higher temperatures with simultaneous decrease in negative values of ΔG^o (Table-3). (11) the value of ΔG^o is calculated. The values of ΔH^o, ΔS^o and ΔG^o are represented in Table-3. The negative sign The value of ΔH^o indicates that the total energy adsorbed in the bond breaking is less compared to the energy used in the bond making. Similar results were reported in literature for removal of Methylene blue by akash kinari coal [22].

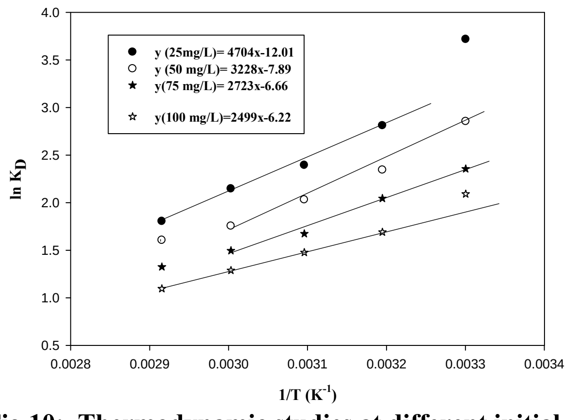

Fig.10: Thermodynamic studies at different initial dye concentrations for biosorption of MB dye onto RR Biosorbent

Central Composite Design for Optimization of biosorption process parameters

RSM is a modeling technique through optimization and regression using independent and dependent variables in biosorption process. The most important is the Central Composite Design (CCD) which optimizes the process parameters. It is done by adding two experimental points along each coordinate axis at opposite sides of the origin and at a length equal to the semi -diagonal of the hyper cube of the factorial design [23]. The new acute values (low and high) for each parameter are summed in the model. The application of statistical experimental design techniques adopted in the biosorption process to attain a high degree of dye removal, closer confirmation of the output response to nominal and target requirements and reduced development time and overall costs [24].

$$\alpha = \left[2^{\frac{m}{4}} \right] \quad (12)$$

The equation (12) is used to calculate the number of variables, which in turn gives the number of test points. In this study, the parameters considered are: contact time (min), solution pH, initial dye concentration (mg/L), temperature (K). As four parameters are considered, $m = 4$ and $\alpha = 2$ obtained from above equation (12). A mathematical statement was used in CCD to calculate the number of test points:

$$N = 2^m + 2m + m_o \quad (13)$$

m_o is the number of central points. Substituting ($m = 4$; $m_o = 2$ and $\alpha = 2$) in equation (13), the value of N=26 obtained from the above equation. The (*Y*) is the biosorption efficiency of MB dye. Information from CCD are subjected to a second-order multiple regression analysis to clarify the behavior of the system using the least squares regression methodology for obtaining the parameter of the numerical model [25].

$$Y = \alpha_o + \sum_{i=1}^{k} \alpha_i x_i + \sum_{i=1}^{k} \alpha_{ii} x^2{}_i + \sum_{i<j} \sum \alpha_{ij} x_i x_j + \in$$

Where Y is the response, α_o is the constant, α_i is the incline or straight impact of the data element x_i, α_{ii} is the quadratic effect of input factor x_i, α_{ij} is the linear by linear interaction effect between the input factor x_i and ϵ is the residual term.

STATISTICA 6.0 software was applied to assess the data and to fit the coefficients in the equation. The surface plots of response were developed to examine the effects of individual and cumulative variables and their dependence. The suitability of regression model was tested by Analysis of variance (ANOVA).

Development of regression model equation

To develop a correlation between biosorption efficiency and process variables affecting the biosorption, a Central Composite Design (CCD) was used. The range of experimental values and levels of independent variables are mentioned in Table 4. A software was selected to fit the quadratic model**.** Experiments of 26 trails were planned to develop a quadratic equation. The results of CCD are shown in Table 4.

Regression analysis is executed to fit the response function of MB dye. The variables are represented as an equation with removal (Y) as a function of contact time (X_1), solution pH (X_2), initial dye concentration(X_3) and temperature(X_4). Maximum biosorption of 99.7% under optimal conditions is represented by the design. From Table-8 a second-order polynomial equation was developed. By using Design expert software, Regression analyses, ANOVA tables response surfaces were developed. The experimental data with multiple regression analysis was obtained from the following regression equation:

%Removal(Y)= $-32047.5+38.6X_1+25.7X_2+5.1X_3+202.3X_4-0.1X_1^2-0.7X_2^2-0.0X_3^2+0.3X_4^2+0.0X_1X_2-0.0X_1X_3-0.1X_1X_4-0.0X_2X_3-0.1X_2X_4-0.0X_3X_4$

Where X_1, X_2, X_3 *and* X_4 are the code values for the independent variables, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4, X_1^2, X_2^2, X_3^2 and X_4^2 are the significant model terms for the adsorption of MB dye. The regression coefficients calculated are represented in Table.8. The biosorption efficiency can be estimated by calculating the coefficient of determination (R^2), standard error, t-values, p-values and Fisher's 'F' test value. The experimental process variables and their interactions give the R^2 value. The value of correlation R^2 is 95.4% (Table-6). Table-5 represents the biosorption efficiency of experimental data versus predicted data. A plot of experimental and the predicted percentage biosorption of MB dye are shown in Fig.14.

Table 4: Experimental variables and their levels

Variable	Process Parameter	Range of Process parameters				
		-2	-1	0	1	2
X_1	Contact Time (min)	65	70	75	80	85
X_2	Solution pH	2	4	6	8	10
X_3	Initial dye concentration (mg/L)	15	20	25	30	35
X_4	Temperature (K)	299	301	303	305	307

Table 5: Experimental values Vs. Predicted Values

S.NO	% Biosorption of Experimental value	% Biosorption of Predicted value
1	90.3200	90.61917
2	91.3500	90.80333
3	91.5300	90.79333
4	90.3700	90.68250
5	95.5200	95.91167
6	94.9600	95.18583
7	94.1300	94.60583
8	93.3700	93.58500
9	92.2200	91.88167
10	87.6700	88.02583
11	90.5600	91.16583
12	87.5300	87.01500
13	97.6300	98.14917
14	92.7700	93.38333
15	95.5300	95.95333
16	90.3600	90.89250
17	94.1700	94.20583
18	93.5200	92.77583
19	82.6100	83.24583
20	93.7600	92.41583
21	96.2500	95.84417
22	93.8300	93.52750
23	96.5600	96.09417
24	91.4600	91.21750
25	98.8000	98.80000
26	98.8000	98.80000

Table 6: Analysis of variance (ANOVA) for response surface quadratic model for removal of MB dye using RR biosorbent

	SS	Df	Mass Square	F	P(prob>F)
X_1	3.0673	1	3.0673	4.9723	0.047537
X_1^2	30.7497	1	30.7497	49.8470	0.000021
X_2	126.1334	1	126.1334	204.4692	0.000000
X_2^2	131.2610	1	131.2610	212.7815	0.000000
X_3	8.0504	1	8.0504	13.0502	0.004080
X_3^2	18.4651	1	18.4651	29.9330	0.000195
X_4	35.6728	1	35.6728	57.8276	0.000011
X_4^2	28.8681	1	28.8681	46.7969	0.000028
X_1X_2	0.9506	1	0.9506	1.5410	0.240286
X_1X_3	0.7921	1	0.7921	1.2840	0.281244
X_1X_4	16.3216	1	16.3216	26.4582	0.000321
X_2X_3	2.1904	1	2.1904	3.5508	0.086202
X_2X_4	0.8281	1	0.8281	1.3424	0.271147
X_3X_4	0.0870	1	0.0870	0.1411	0.714359
Error	6.7857	11	0.6169		
Total SS	333.8610	25	R^2 =.97968; R^2(Adj)=95.4		

DF: degree of freedom; SS: sum of squares; F: factor F; P: probability.

Table 7: Optimized process parameters

Process parameters	Initial observed limits of process parameters	Critical values of process parameters	Final observed limits of process parameters
Contact time (min)	65	75.7728	85
Solution pH	2	6.9457	10
Initial dye concentration (mg/L)	15	23.1849	35
Temperature (K)	299	301.8678	307

99.7% is the predicted value of biosorption efficiency of MB dye on RR biosorbent using CCD

Effect of process variables on biosorption efficiency using RSM

The different combinations of process variables are combined using statistically designed experiments in order to identify the effects of the factors (Table-7). The linear effect of parameters on biosorption efficiency of MB dye along with squared and interaction effects of variables are studied in RSM. These tests were conducted by means of Fisher's *F*- test and Student *t*-test. The Student *t*-test was used to study the importance of regression coefficients of the process parameters. In general, the larger the magnitude of 't' and smaller the value of '*P*'; the more significant is the corresponding coefficient term [26].

The squared effects of X_1^2, X_2^2, X_3^2 and X_4^2 variables for MB dye were also highly important (Table 8), because other P-values are less than 0.05. The positive or negative influence on the dependent variable is identified by the t-value. The response surface plots represent an interaction between the variables. The elliptical shape of the response surface curve indicates good interaction of two variables. Fig.15 (a - f) are plotted between X_1 and X_2, X_1 and X_3, X_1 *and* X_4, X_2 and X_3, X_2 and X_4 ,X_3 and X_4 respectively. The response surface plot (Fig. 15 (a) to 15(f)) had a clear peak, where the optimum conditions falls inside the design boundary.

Table 8: **Regression coefficients and corresponding *t*- and *P*- values of the models.**

	Regression coefficient	SE coefficient	t-value	p-value
constant.	-32047.5	4363.261	-7.3448	0.000015
X_1	38.6	6.060	6.3650	0.000053
X_1^2	-0.1	0.008	-7.0602	0.000021
X_2	25.7	14.965	1.7175	0.113874
X_2^2	-0.7	0.047	-14.5870	0.000000
X_3	5.1	5.992	0.8454	0.415898
X_3^2	-0.0	0.008	-5.4711	0.000195
X_4	202.3	28.525	7.0929	0.000020
X_4^2	-0.3	0.047	-6.8408	0.000028
X_1X_2	0.0	0.020	1.2414	0.240286
X_1X_3	-0.0	0.008	-1.1332	0.281244
X_1X_4	-0.1	0.020	-5.1438	0.000321
X_2X_3	-0.0	0.020	-1.8843	0.086202
X_2X_4	-0.1	0.049	-1.1586	0.271147
X_3X_4	-0.0	0.020	-0.3756	0.714359

Prediction of the responses at the optimum set of conditions

The optimum biosorption conditions determined using RSM for the biosorbent RR under batch studies are t=75.7 min, *pH*=6.94, C_o= 23.18 mg/L, and *T*=301.8 K. The model accomplishes a maximum biosorption efficiency of 99.7%. By conducting experiment the biosorption efficiency of 98.8% was obtained with optimum process parameters

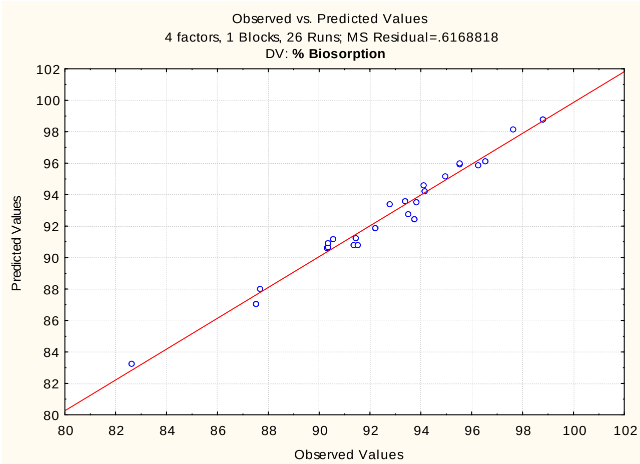

Fig.14: Correlation plot of experimental values Vs predicted values for the biosorption of MB dye.

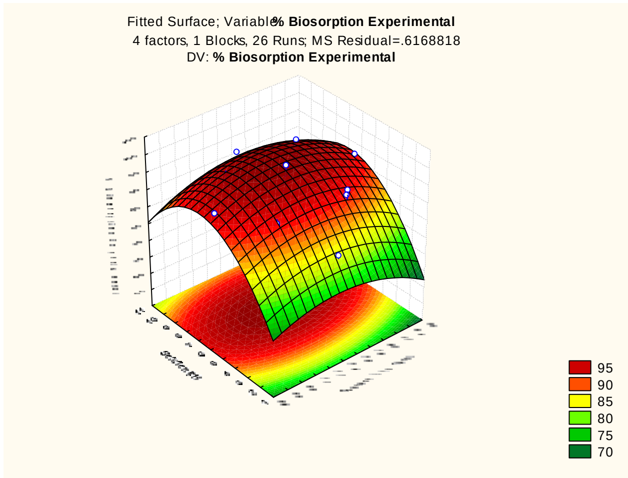

Fig: 15 (*a*). Surface plot of Contact time Vs Solution pH for the biosorption of MB dye onto RR biosorbent.

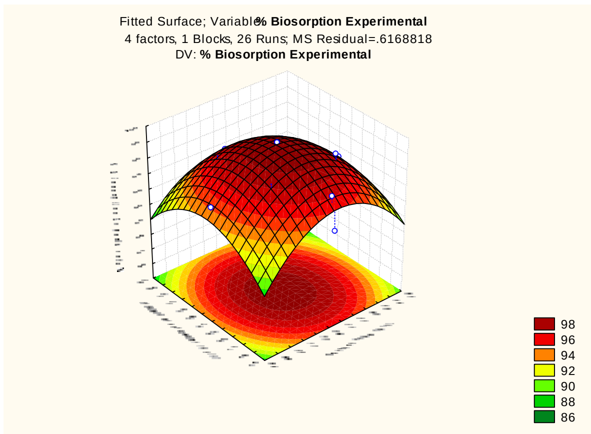

Fig: 15(*b*). Surface plot of Contact time Vs Initial concentration for the biosorption of MB dye onto RR biosorbent.

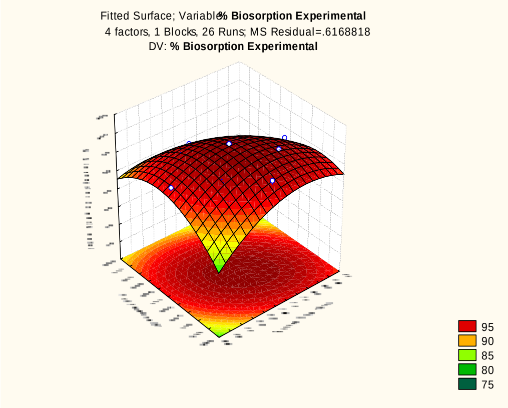

Fig: 15(*c*). Surface plot of Contact time Vs. Temperature plot for thebiosorption of MB dye onto RR biosorbent.

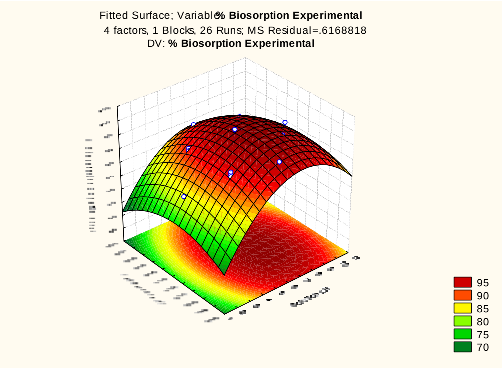

Fig: 15(*d*). Surface plot of Solution pH Vs Initial concentration for the biosorption of MB dye onto RR biosorbent

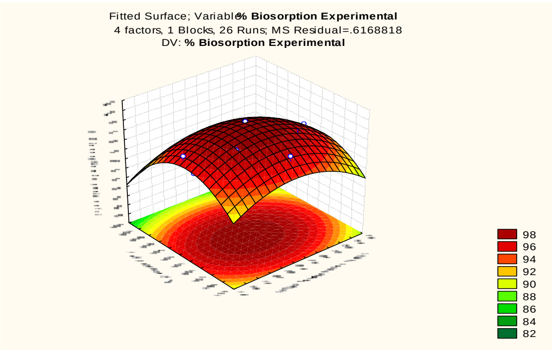

Fig: 15 (*e*). Surface plot of Solution pH Vs Temperature for the biosorption of MB dye onto RR Biosorbent

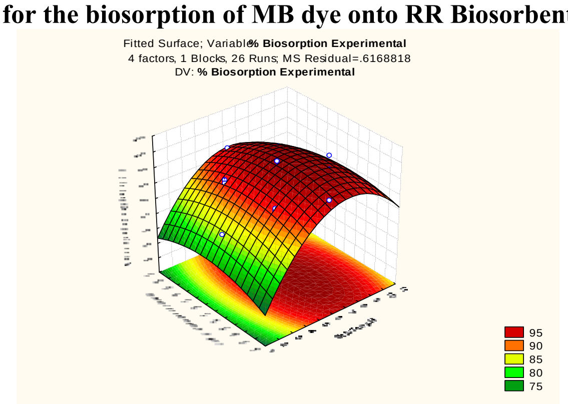

Fig: 15(*f*). Surface plot of Initial concentration Vs Temperature for the biosorption of MB dye onto RR Biosorbent

5. CONCLUSIONS

- The present study shows that the Raphanus Raphanistrum can be used as biosorbent for the removal of methylene blue from an aqueous solution.
- The amount of dye adsorbed was found vary with initial dye concentration, contact time, solution pH, average biosorbent size, biosorbent dosage and temperature.
- The amount of percentage biosorption increased with an increase of contact time, solution pH and biosorbent dosage and found to decrease with an increase of average biosorbent size and temperature.
- The experiment was conducted at contact time =75 min, solution pH= 6. Initial dye concentration= 25mg/L, biosorbent dosage=0.1g, temperature = 303K and average biosorbent size=63-212µm, where 98.8% of maximum biosorption efficiency of MB dye onto RR biosorbent was obtained.
- Equilibrium data were fitted to Freundlich isotherm equation.
- The kinetics of the MB dye onto RR biosorbent has been described by second order rate constant.
- The negative sign values of thermodynamic parameters ΔG^o, ΔH^o and ΔS^o gives the process is exothermic in nature and as the efficiency of biosorption process is increased, the deposition of MB dye onto RR biosorbent surface takes place at lower temperatures.
- The RSM outcomes that were analyzed statistically under the optimum conditions are identified as contact time=75.7min, solution *pH*= 6.94, initial dye concentration = 23.18 mg/L, and temperature= 301.8K. By using CCD, the predicted value of biosorption efficiency is 99.7%.
- The response surface plots describes that the efficiency of biosorption is influenced highly by the independent variables like contact time, solution pH, initial dye concentration and temperature of the biosorbent.

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