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STUDIES ON BIOSORPTION OF METHYL RED DYE WITH SARGASSUM VULGARE POWDER AND OPTIMIZATION THROUGH CENTRAL COMPOSITE DESIGN

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Abstract: ***The major three resources like Air, Water and Food have been polluted and are seeking a special attention for their originality which has to be reestablished. The present research was investigated using Sargassum vulgare powder as a potential biosorbent for the removal of Methyl red. The operating parameters involved are agitation time, biosorbent size, pH of the solution, initial concentration of solution, dosage of biosorbent and temperature of the solution. The optimization was also incorporated using Central Composite Design (CCD). The optimum size of biosorbent is 53 μm, pH was obtained at 6.0 and initial concentration of MR is 20 mg/L were compared using one factor at a time with CCD. The kinetics and isotherm studies are also studied along with thermodynamic study.***

Keywords: MR, Sargassum vulgare, RSM, CCD.

1. Introduction

Issues concerning water and its pollution are current challenges mankind is facing [1]. Industrialization has led to generation of waste water. Wastewater generated is the major source of water pollution [2]. Water is considered most crucial of the natural resources gifted to human life yet being polluted due to never ending human activities out of which urbanization and industrialization have greater impact on its pollution. with the increase in extent of dependence of man on industrialization pollution of natural resources in increasing at an alarming rate. Various pollution control strategies and wastewater treatment methods are in use today to treat wastewater effectively and restore water quality [3,4,5]. Since wastewater includes various contaminants to treat, we are finding it challenging to treat those wastewaters containing organic content and color. [7] Above all, though various techniques have been developed and employed, all of them are not desirable with respect to cost considerations. Conventional treatment methods are now replaced by novel low-cost techniques [5]. Apart from cost considerations there are various other limitations such as generation of sludge etc. [6]. On analysis of various cost-efficient methods available biosorption is discovered to be a novel low-cost route. It has been satisfactory both in terms of efficiency and cost effectiveness to solve the above problem. The present experimentation was carried out in order to evaluate the potential and power of red algae powder (Sargassum vulgare) for the removal of novel dye MR dye for the first time.

2.0 EXPERIMENTAL PROCEDURE

The current experiments are conducted in a batch mode that focus on the biosorption process of Methyl red dye by using two different biosrobents such as Sargassum vulgare powders, from the waste waters.
The procedure of the experiments is divided into the below mentioned steps:
2.1. Preparation of the Biosorbent
2.2. Preparations of the 1000 mg/L of MR dye stock solution.
2.3. Studies on Equilibrium Biosorption Process.

2.1 Preparation of the Biosorbent

Sargassum vulgare algae were collected from Jodugullapalem beach in Visakhapatnam and were washed with water to remove dust and soluble impurities and dried in sun light till the algae became crispy and colorless. By passing it through a set of sieves ranging from 300 to 75 mesh sizes the dried algae were finely powdered and sized. The powder of 53, 75, 105, 125 and 152 micron meters were separated and stored in dry bottles with double cap and used as biosorbent.

2.2 Preparation of 1000 Mg/L of MR Dye Stock Solution

To prepare 1000 ppm of MR dye stock solution 1.0 g of 99 % MR dye powder was dissolved in 1.0 L of distilled water. From this stock solution synthetic samples of different concentrations of MR dye were prepared by appropriate dilutions. 100 ppm MR dye stock solution was prepared by diluting 100 ml of 1000 ppm MR stock solution with distilled water in 1000 ml volumetric flask up to the mark. Similarly solutions with different dye concentrations such as 20, 50, 100, 150 and 200 ppm were prepared.

2.3 Studies on Equilibrium Biosorption Process

The biosorption was carried out in a batch process by adding a pre-weighed amount of the Sargassum vulgare algae powder to a known volume of aqueous solution for a predetermined time interval in an orbital shaker. The procedures adopted to evaluate the effects of various parameters via. Agitation time, biosorbent size, pH, initial concentration, biosorbent dosage and temperature of the aqueous, which include characterization (FTIR, XRD, SEM), Isotherms (Langmuir, Freundlich, Temkin), Kinetics (Lagergren First Order, Pseudo Second Order), Thermodynamics (Entropy, Enthalpy and Gibb's Free Energy) and Optimization using Central Composite Design

3.0 RESULTS AND DISCUSSION

Biosorption of MR dye onto sargassum vulgare powder

Biosorption of MR dye using sargassum vulgare powder has many affecting factors which include characterization (FTIR, XRD, SEM), equilibrium studies (agitation time, biosorbent size, pH, initial concentration, biosorbent dosage, temperature), Isotherms (Langmuir, Freundlich, Temkin), Kinetics (Lagergren First Order, Pseudo Second Order), Thermodynamics (Entropy, Enthalpy and Gibb's Free Energy) and Optimization using Central Composite Design.

3.1 Characterization of sargassum vulgare powder

3.1.1 (a) FTIR spectrum of untreated Sargassum vulgare powder

FTIR measurements presented in fig. 3.1.1(a) show the broad band at 3544.35 cm-1 is due to stretching adsorption of –OH. The band at 2932.89 cm-1 denote the presence of stretching C–H vibrations in CH2 or C = C – H group. The band at 1616.42 cm-1 suggests the presence of stretching C=C aromatic rings. The band at 3249.23 cm-1 is may be due to the stetching vibration bands of Hydroxyl bonds. Asymmetric stretching vibration of C=O is due at 1653.07 cm-1 band.

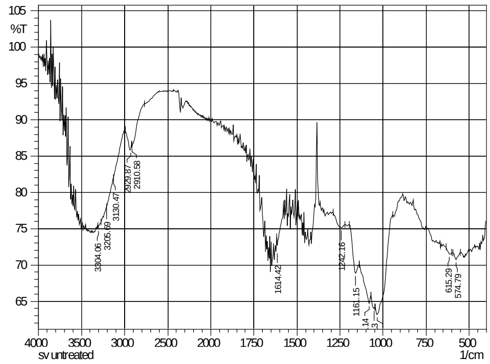


Fig. 3.1.1 (a) FTIR spectrum of untreated sargassum vulgare powder

3.1.1(b) FTIR spectrum of treated sargassum vulgare powder

FTIR spectrum for treated powder is shown in fig 3.1.1(b). Broad band at 3566.53 cm-1 suggests stretching adsorption of O–H. The band at 3266.59 cm-1 due to the presence of stretching C–H vibrations in CH2 or C = C – H group. The band at 1653.07 cm-1 indicates the presence of asymmetric stretching C=O vibrations arising from group such as lactone, quinine and carboxylic acids. The stretching vibration of C–N and stretching of C=C aromatic rings, respectively appear at 1457.28 cm-1, 1396 cm-1 bands. The vibration at 1244.14 cm-1 be attributed to –SO3 stretching bond.

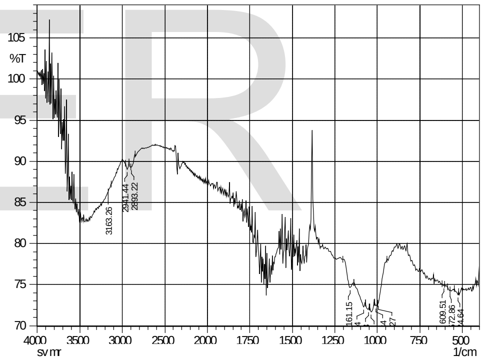


Fig. 3.1.1 (b) FTIR spectrum of treated sargassum vulgare powder

3.1.2 (a) X–Ray Diffraction for untreated sargassum vulgare powder

XRD patterns shown in fig 3.1.2.1(a)(b) for untreated powder do not show very acute or keen and discrete peaks and exhibits minimum amorphous nature. The peaks at 2θ values of 0.3845, 0.6273, 0.5076, 0.6547 and 0.4937 corroborate the presence of NP3O13Se3, O2Si, Rb12Si17, AuCs and Al1.65Na1.65O4Si0.35. Their corresponding d-values are 4.1049, 4.2516, 3.8633, 3.0189 and 2.5648.

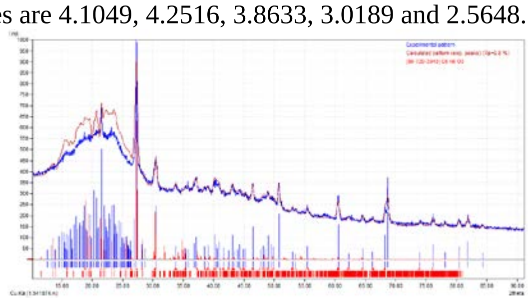


Fig. 3.1.2.1 (a) XRD pattern of untreated sargassum vulgare powder

Fig. 3.1.2.1 (b) XRD pattern of MR dye untreated sargassum vulgare powder with matching compounds

3.1.2(b) X–Ray Diffraction for treated sargassum vulgare powder

XRD patterns of treated MR dye, shown in figs 3.1.2.2(a)(b), show very spiky and clear peaks and exhibit absolutely amorphous nature. The peaks at 2θ values of 0.4584, 0.6664, 0.5909, 0.6593 and 0.6948 corroborate the presence of Ni(HN2S2)2, O2Si, CCaO3, Cs23O14.15 and C (graphite). Their corresponding d-values are 1.8171, 2.2843, 3.0168, 2.9714 and 2.1314 respectively.

Fig. 3.1.2.2 (a) XRD pattern of treated sargassum vulgare powder

Fig. 3.1.2.2 (b) XRD pattern of MR dye treated Sargassum vulgare powder with matching compounds

3.1.3 Scanning Electron Microscope (SEM):

3.1.3 (a) Scanning Electron Microscope for untreated sargassum vulgare powder

The SEM micrographs of sargassum vulgare powder before and after biosorption are analyzed. The SEM images in fig. 3.1.3 (a) show that the algae powder is not at all even and lots of pores are available.

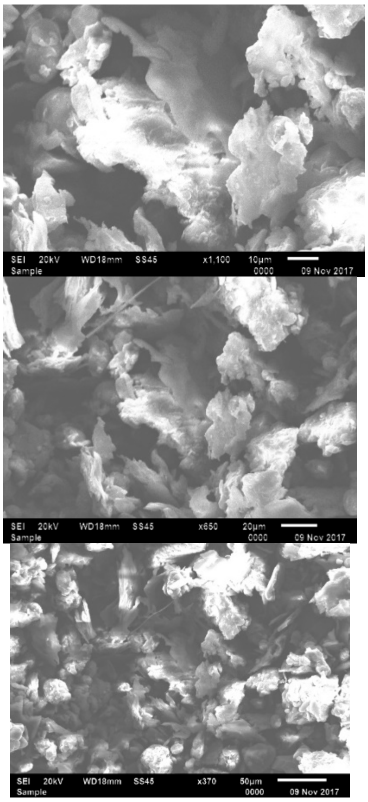
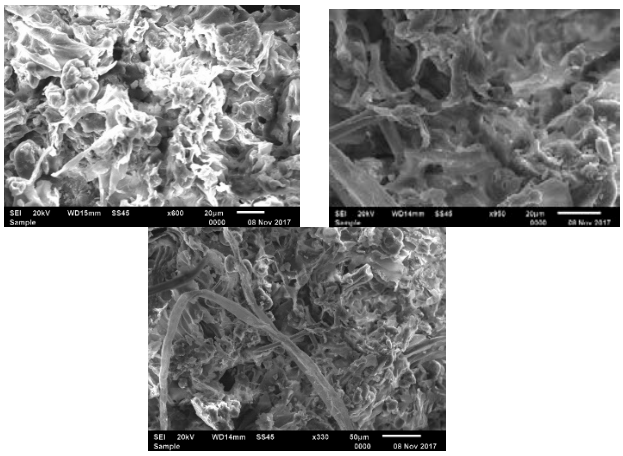


Fig. 3.1.3 (a) Electron micrographs of untreated sargassum vulgare powder

3.1.3 (b) Scanning Electron Microscope for treated Sargassum vulgare powder

The micrographs presented shows clearly the dye loaded biosorbent coated by dye molecules over the whole surface. The dye molecules seem to have formed a void-free film masking the reliefs of particles and porosity of the aggregates.



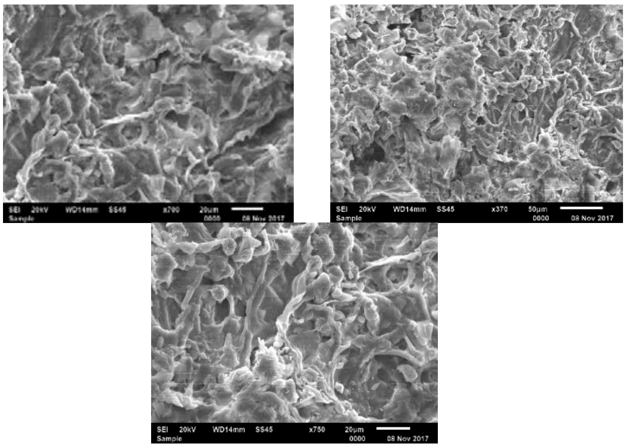


Fig. 3.1.3 (b) Electron micrographs of treated sargassum vulgare powder

3.2 Equilibrium studies on biosorption:

3.2.1 Effect of agitation time:

The equilibrium agitation time is determined by plotting the % biosorption of MR dye against agitation time as shown fig. 3.2.1 for the interaction time intervals between 5 to 180 min. Beyond 25 min, the % biosorption is constant indicating the attainment of equilibrium conditions [8-17].

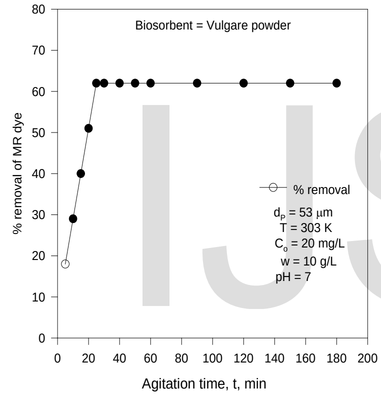


Fig. 3.2.1 Effect of agitation time on % biosorption of MR dye

3.2.2 Effect of biosorbent size:

The results are drawn in fig. 3.2.2 with percentage biosorption of MR dye as a function of biosorbent size. The percentage biosorption is increased from 32 % to 62 % as the biosorbent size decreases from 152 to 53 μm. [18-27].

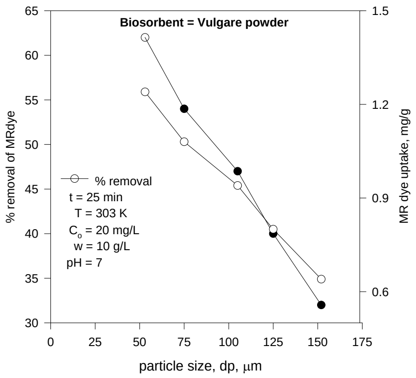


Fig. 3.2.2 % Biosorption of MR dye as a function of biosorbent size

3.2.3 Effect of pH:

The effect of pH of aqueous solution on % biosorption of MR dye is shown in fig. 3.2.3. The % biosorption of MR dye is increased drastically from 50% to 70 % as pH is increased from 2 to 6 [28-37]and beyond the pH value of 6 it increased slowly and the margin is also very less.

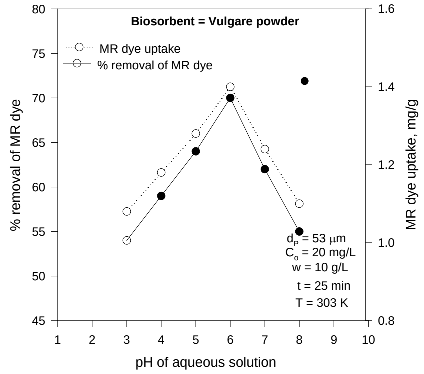


Fig. 3.2.3 Observation of pH along with % biosorption of MR dye

3.2.4 Effect of initial concentration of MR dye:

The effect of initial concentration of MR dye in the aqueous solution on the percentage biosorption of MR dye is shown in fig. 3.2.4 The percentage biosorption of MR dye is decreased from 70 % to 48 % with an increase in C0 from 20 mg/L to 200 mg/L [38-47].

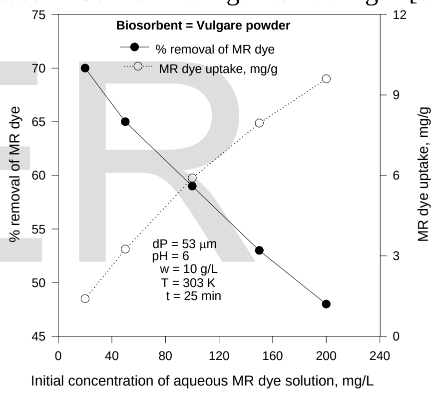


Fig. 3.2.4 Variation of initial concentration with % biosorption of MR dye

3.2.5 Effect of biosorbent dosage:

The percentage biosorption of MR dye is drawn against biosorbent dosage for 53 μm size biosorbent in fig. 3.2.5. The biosorption of MR dye increased from 70 % to 90 % with an increase in biosorbent dosage from 10 to 40 g/L [48-57]. The change in percentage biosorption of MR dye is marginal from 90 % to 91.5 % when dosage is increased from 40 to 80 g/L. Hence all other experiments are conducted at 40 g/L dosage.

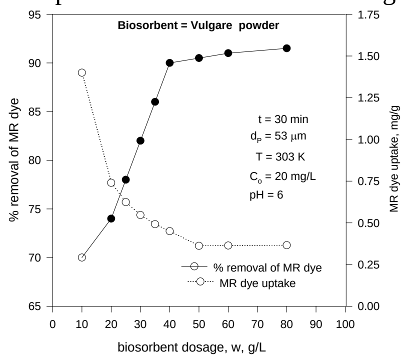


Fig. 3.2.5 Dependency of % biosorption of MR dye on biosorbent dosage

3.2.6 Effect of Temperature:

The effect of temperature on the equilibrium dye uptake was significant. The effect of changes in the temperature on the MR dye uptake is shown in Fig. 3.2.6. High temperature favors the diffusion of dye molecules in the internal porous structure of surface [58-67].

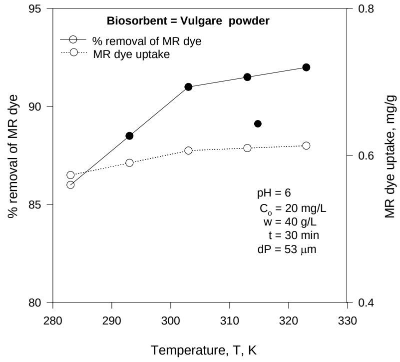


Fig. 3.2.6 Effect of temperature on % biosorption of MR dye

3.3 Isotherms:

3.3.1 Langmuir isotherm:

Langmuir isotherm is drawn for the present data and shown in Fig. 3.3.1. The equation obtained is: Ce/qe = 0.06586 Ce + 4.1156 -----(1)with a good linearity (correlation coefficient, R2~0.9960) indicating strong binding of MR dye to the surface of sargassum vulgare powder.[68-77]

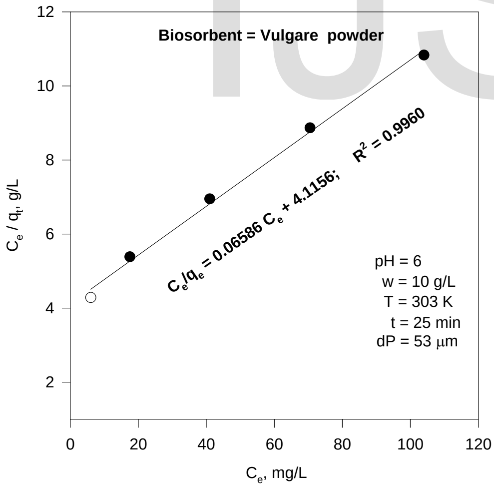


Fig. 3.3.1 Langmuir isotherm for % biosorption of MR dye

3.3.2 Freundlich isotherm:

Freundlich isotherm is drawn between ln Ce and ln qe and is shown in Fig. 3.3.2 for the present data. The resulting equation has a correlation coefficient of 0.9929.

ln qe = 0.6797 ln Ce - 0.8224 ------(2)

The 'n' value in the above equation (n=0.6797) satisfies the condition of 0<n<1 indicating favorable biosorption. [78-87].

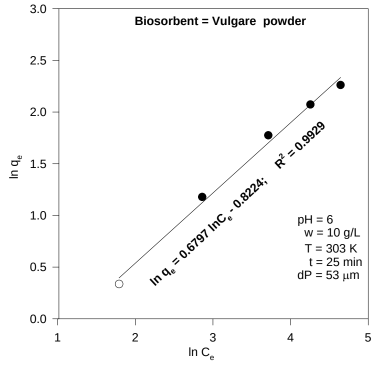


Fig. 3.3.2 Freundlich isotherm for % biosorption MR dye

3.3.3 Temkin isotherm:

The present data are analysed according to the linear form of Temkin isotherm and the linear plot is shown in Fig. 3.3.3. The equation obtained for MR dye biosorption is: qe = 2.5276 ln Ce – 3.0036 -----(3) with a correlation coefficient 0.9834. From the Figs 3.3.1, 3.3.2 & 3.3.3, it is found that biosorption data are well represented by Langmuir isotherm with higher correlation coefficient of 0.9960, followed by Temkin and Freundlich isotherms with correlation coefficients of 0.9929 and 0.9712 [88-97] respectively.

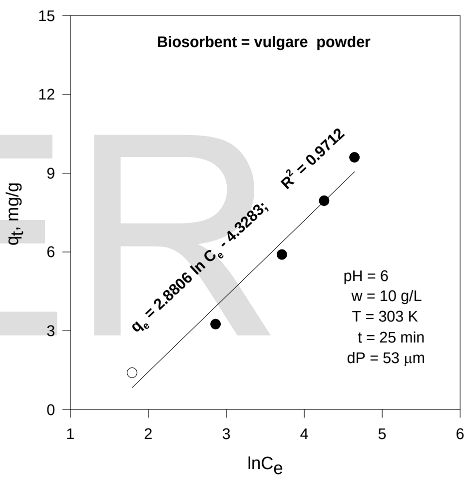


Fig. 3.3.3 Temkin isotherm for % biosorption of MR dye

3.4 Kinetics of biosorption

3.4.1 Lagergren First order Kinetics

In the present study, Lagragen plots of log (qe-qt) versus agitation time (t) for biosorption of MR dye the biosorbent size (53 µm) of sargassum vulgare powder in the interaction time intervals of 5 to 180 min are drawn in fig. 3.4.1.[98-107].

log (qe-qt) = 0.0427 t – 0.0162 ------(4)

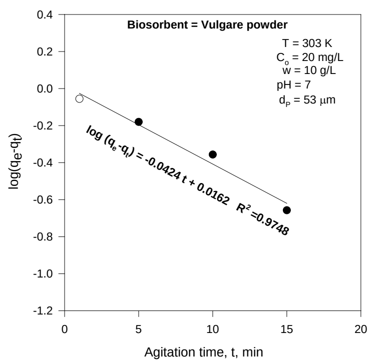


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Fig. 3.4.1 first order kinetics for % biosorption of MR dye

3.4.2 Pseudo Second order Kinetics

Pseudo second order plot of t vs t/qt for biosorption of MR dye with the biosorbent size (53 µm) of sargassum vulgare powder in the interaction time intervals of 5 to 180 min is drawn in fig. 3.4.2. [108-117].

$t/qt = 0.3898\ t + 14.3503$ ------(5)

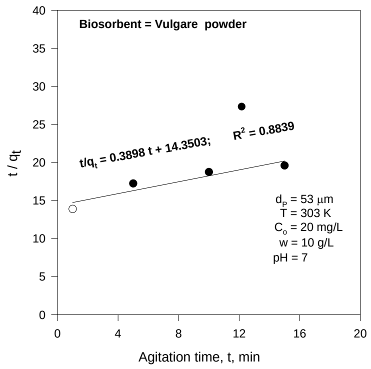

Fig. 3.4.2 second order kinetics for % biosorption of MR dye

3.5 Thermodynamics of biosorption:

$\log (qe/Ce) = -0.6374\ (1/T) + 1.5857$ …….. (6)

Where (qe/Ce) is called the biosorption affinity.
initial MR dye concentrations is shown in fig. 3.5. The values are ΔG = -9187.37, ΔH = 12.20439 and ΔS = 30.3616 [118-127].

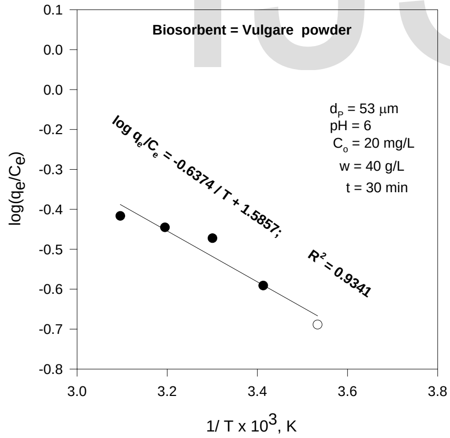

Fig 3.5 Vantoff's plot for % biosorption of MR dye

3.6. Optimization using Response Surface Methodology (RSM):

3.6.1 Optimization of biosorption conditions using CCD

The effects of four independent variables (pH, initial concentration of MR dye in aqueous solution, biosorbent dosage and temperature) on MR dye biosorption are analyzed using Central Composite Design (CCD) [128-137]. Levels of different process variables for percentage biosorption are shown in table–1.

Table–1: Levels of different process variables in coded and un-coded form for % biosorption of MR dye using sargassum vulgare powder

Variable	*Name*	*Range and levels*				
		-2	*-1*	*0*	*1*	*2*
X1	pH of aqueous solution	4	5	6	7	8
X2	Initial concentration, Co, mg/L	10	15	20	25	30
X3	Biosorbent dosage, w, g/L	20	25	30	35	40
X4	Temperature, T, K	283	293	303	313	323

Regression equation for the optimization of biosorption is:
% biosorption of MR dye (Y) is function of pH of aqueous solution (X1), initial concentration (X2), dosage (X3), and Temperature of aqueous solution (X4).

The multiple regression analysis of the experimental data has yield the following equation:

$Y = -3869.31 - 66.06\ X1 + 7.12\ X2 + 4.04\ X3 + 23.75\ X4 - 5.62\ X12 - 0.19X22 - 0.04X32 - 0.04\ X42 + 0.05\ X1X2 - 0.01\ X1X3 - 0.00\ X1X4 - 0.000\ X2X3 - 0.00\ X2X4 - 0.00\ X3X4$ ---- (7)

Table-6.5 represents the results obtained in CCD. The response obtained in the form of analysis of variance (ANOVA) from regression eq. 7 is put together in table–2. Fischer's 'F-statistics' value is defined as MSmodel/MSerror, where MS is mean square. Fischer's 'F-statistics' value, having a low probability 'p' value, indicates high significance.

Table–2: Results from CCD for MR dye biosorption by sargassum vulgare powder

Run No.	*X1, pH*	*X2, Co*	*X3, w*	*X4, T*	*% biosorption of MR dye*	
					Experimental	*Predicted*
1	5	15	20	293	73.5800	73.57167
2	5	15	20	313	75.7200	75.71917
3	5	15	30	293	75.5200	75.52250
4	5	15	30	313	76.5200	76.53500
5	5	25	20	293	71.3800	71.39250
6	5	25	20	313	73.4200	73.40500
7	5	25	30	293	73.4200	73.40833

8	5	25	30	313	74.2800	74.28583
9	7	15	20	293	74.2600	74.26917
10	7	15	20	313	76.5200	76.52167
11	7	15	30	293	76.5000	76.50500
12	7	15	30	313	77.6200	77.62250
13	7	25	20	293	73.1200	73.09500
14	7	25	20	313	75.2000	75.21250
15	7	25	30	293	75.3800	75.39583
16	7	25	30	313	76.3800	76.37833
17	4	20	25	303	69.1200	69.12250
18	8	20	25	303	71.9200	71.91250
19	6	10	25	303	76.2200	76.20917
20	6	30	25	303	72.7800	72.78583
21	6	20	15	303	75.6800	75.68917
22	6	20	35	303	78.8200	78.80583
23	6	20	25	283	75.8800	75.88250
24	6	20	25	323	79.0200	79.01250
25	6	20	25	303	93.0000	93.00000
26	6	20	25	303	93.0000	93.00000
27	6	20	25	303	93.0000	93.00000
28	6	20	25	303	93.0000	93.00000
29	6	20	25	303	93.0000	93.00000
30	6	20	25	303	93.0000	93.0000

Experimental conditions [Coded Values] and observed response values of central composite design with 24 factorial runs, 6- central points and 8- axial points. Agitation time fixed at 25 min and biosorbent size at 53 µm

Table–3:ANOVA of MR dye biosorption for entire quadratic model

Source of variation	*SS*	*df*	*Mean square(MS)*	*F-value*	*P > F*
Model	1691.418	14	120.8155	604077	0.00000
Error	0.003	15	0.0002		
Total	1691.421				

Df- degree of freedom; SS- sum of squares; F- factor F; P- probability.
R^2=0.99996; R^2 (adj):0.99992

Table-4: Estimated regression coefficients for the MR dye biosorption onto sargassum vulgare powder

Terms	*Regression coefficient*	*Standard error of the coefficient*	*t-value*	***P*-value**
Mean/Intercept	-3869.31	2.461520	-1571.92	0.000000
Dosage, w, g/L (L)	66.06	0.105196	627.97	0.000000
Dosage, w, g/L (Q)	-5.62	0.002498	-2250.33	0.000000
Conc, Co, mg/L (L)	7.12	0.020766	342.86	0.000000
Conc, Co, mg/L (Q)	-0.19	0.000100	-1851.97	0.000000
pH (L)	4.04	0.010383	388.99	0.000000
pH (Q)	-0.04	0.000025	-1576.71	0.000000
Temperature, T, K (L)	23.75	0.015377	1544.65	0.000000
Temperature, T, K (Q)	-0.04	0.000025	-1556.69	0.000000
1L by 2L	0.05	0.000654	76.83	0.000000
1L by 3L	0.01	0.000327	21.79	0.000000
1L by 4L	0.00	0.000327	8.03	0.000001
2L by 3L	0.00	0.000065	4.97	0.000168
2L by 4L	-0.00	0.000065	-10.32	0.000000
3L by 4L	-0.00	0.000033	-86.77	0.000000

ainsignificant ($P \geq 0.05$)

The ANOVA of the regression model is sufficiently great, as proven from the Fisher's F-test and has a very low probability value (Pmodel > F=0.000000). Besides, the computed F-value is much higher compared to F-value ($F_{0.05}$ (14.15) tabulars = 2.42) at 5% level, suggesting that the treatment differences are sufficiently great. Student's t-test can implicate regression coefficient of the parameter, while pattern of interactions amidst all the factors can be entailed by 'p' values. It is noted from table-4 that more significant corresponding coefficient term can be possessed by having high't' value and low 'P' value. By analyzing't' and 'p' values from table-4, all the variables have high importance to explain the individual and interaction effects of independent variables on biosorption of MR dye to anticipate the response. The model is reduced to the following form by excluding undistinguished terms in eq.2.

$Y = -3869.31 - 66.06 X1 + 7.12 X2 + 4.04 X3 + 23.75 X4 - 5.62 X12 - 0.19X22 - 0.04X32 - 0.04 X42 + 0.05 X1X2 - 0.01 X1X3$ ------- (8)

A positive sign of the coefficient represents an interactive effect i.e., response (% biosorption of MR dye) steps up with increase in effect, whereas a negative sign implies an incompatible effect that means response lowers with an increase in effect.

Measure of the model's variability to the responses indicated is presented by correlation coefficient (R2). As R2 —> 1, model is inviolable and the response is estimated better. In our study, R2 = 0.99996 suggests that 0.004 % of the total variations are not adequately explained by the model. Statistical relevance of the ratio of mean due to regression and mean square due to residual error is tested with the help of ANOVA. F-values implicate that % biosorption can be sufficiently explained by the model equation. If 'P' value is lower than 0.05, the model is considered to be statistically significant at the 95 % confidence level (fig 3.6.1).

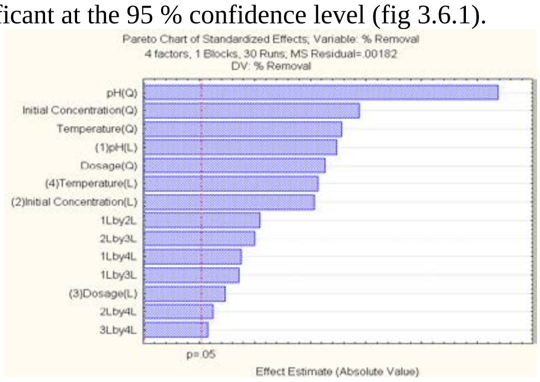

Fig. 3.6.1 Pareto Chart

3.6.2 Interpretation of residual graphs:

Normal probability plot (NPP) is a graphical technique used for analyzing whether or not a data set is normally distributed to greater extent. Fig. 3.6.2 exhibits normal probability plot for the present data. It is evident that the experimental data are reasonably aligned implying normal distribution.

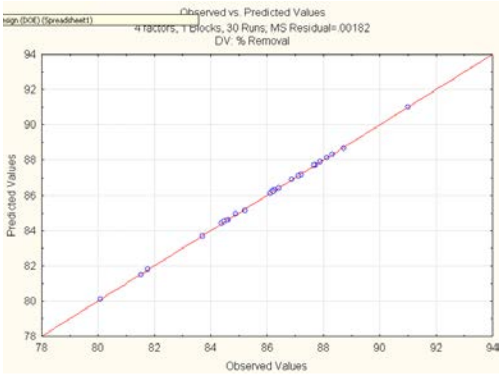

Fig. 3.6.2 Normal probability plot for % biosorption of MR

3.6.3 Interaction effects of biosorption variables:

Three-dimensional view of response surface contour plots [Fig. 3.6.3 (a) to 3.6.3(f)] exhibit % biosorption of the MR dye using sargassum vulgare powder for different combinations of dependent variables.

The predicted optimal set of conditions for maximum % biosorption of MR dye is:

pH of aqueous solution = 6.0608
Initial MR dye concentration = 19.5448 mg/L
Biosorbent dosage = 40.9577g/L
Temperature = 303.9773 K
% biosorption of MR = 93.13573

The experimental optimum values are compared with those predicted by CCD in table-5. The experimental values are in close agreement with those from CCD. The uptake capacities for various other biosorbents were compared and presented in table-6.

Table-5: Comparison between optimum values from CCD and experimentation

Variable	*CCD*	*Experimental*
pH of aqueous solution	6.0608	6.0
Initial MR dye concentration, mg/L	19.5448	20
Biosorbent dosage, w, g/L	40.9577	25
Temperature, K	303.9773	303
% biosorption	93.13373	90

Table – 6 : Dye uptake capacities for different biosorbents

Authors	*Biosorbent*	*qt, mg/g*
Gupta et al. [138]	Spirogyra sp.	140.84
Flavio et al. [139]	Ponkan peel	112.1
Ruhan et al. [140]	Lactarius scrobiculatus	56.2
Matheickal et al. [141]	Powder activated carbon	20.7
Lijuan Wang et al [142]	Crofton weed stalk	28
Present investigation	sargassum vulgare powder	**15.18382**

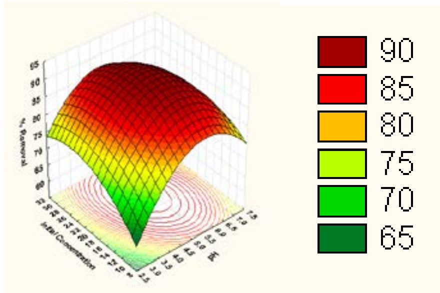

Fig. 3.6.3 (a) Surface contour plot for the effects of pH and initial concentration of MR on % biosorption

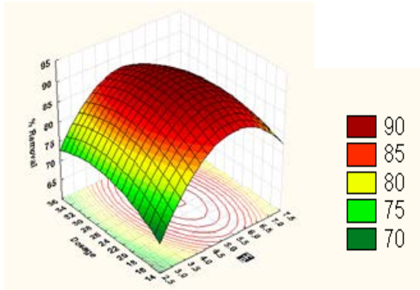

Fig. 3.6.3 (b) Surface contour plot for the effects of pH and Dosage of MR in aqueous solution on % biosorption

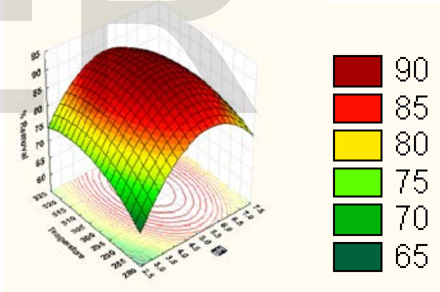

Fig. 3.6.3 (c) Surface contour plot for the effects of pH and temperature of MR dye in aqueous solution on the % biosorption

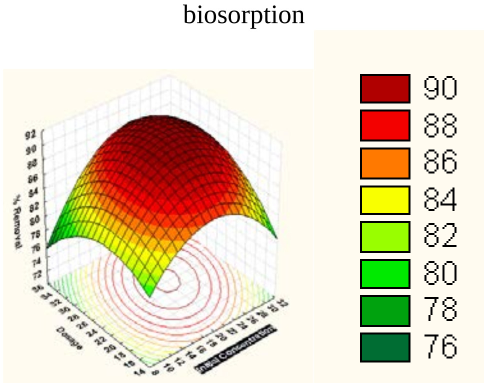

Fig. 3.6.3 (d) Surface contour plot for the effects of concentration and dosage on % biosorption of MR dye

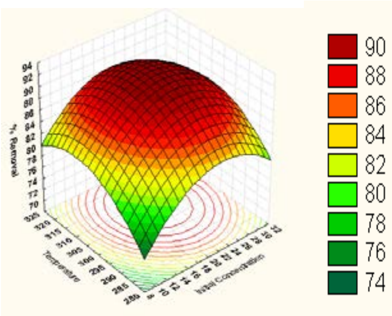

Fig. 3.6.3 (e) Surface contour plot for the effects of concentration and temperature on % biosorption of MR dye

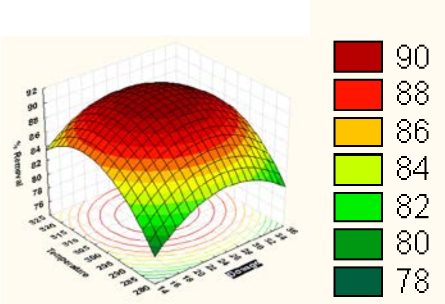

Fig. 3.6.3 (f) Surface contour plot for the effects of Dosage and temperature on % biosorption of MR dye

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