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Congo Red Dye Removal using Hydrilla Verticillata Powder and Optimization through Box Behnken Design

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Abstract: *Dyes are considered to be one of the most dangerous effluents of industries causing environmental hazards as well as health hazards. This paper enhances the treatment technique of biosorption for industrial waste waters containing congo red dye. The bio material considered for this study is a fresh water algae (hydrilla verticillata). As to identify the character of the hydrilla, studies of FTIR, XRD and SEM were undergone. The process is carried out through different parameters like agitation time varying between 1minute and 180 minutes, pH ranging from 2 to 8, Initial concentration of CR dye varies between 20mg/L and 200 mg/L, biosorbent dosages start from 10g/L to 60g/L and temperatures lie in between 283K and 323 K. The study takes account of kinetics, isotherms as well as thermodynamic studies. Optimization process is done through BOX BEHNKEN design. Biosorption is prominent at the conditions of 40minutes of time, pH of 5, dosage of the adsorbent as 40g/L, and initial concentration 20mg/L. Of all the three isotherms Freundlich isotherm has a high correlation factor and suits well for the procedure. Pseudo second order kinetics is best fitted for the test.*

Keywords: *Biosorption, Congo red, Hydrilla verticillata, FTIR, XRD, SEM, BBD.*

I. INTRODUCTION

The survival of life depends on water. Water is capable of regulating the temperatures of our planet. Water has also proven its importance for the betterment of life as all the civilisation starts near a water. Along with the civilization the necessities of mankind too increased directing to make the first move towards establishing industries. As the industries increased, the rates of discharge of wastes are also increased, thus creating pollution. The pollution also includes water, creating a disruption for environment along with the human health. Water helps us to live by maintaining the metabolism, the blood flow rates throughout the body and digestion. Water has a tendency to carry the minerals, nutrients and chemicals along with it as a dissolved or suspended matter. Environmental pollution sets off the balance in ecosystem, and health hazards include chronic diseases, and mutated genes sometimes even result in death. As per some water-facts a rough estimation of about 780 million people does not have any access to clean water bodies. The water consumption rate was found to be more than doubled when compared to the rate at which the population is growing during the last century (<http://www.seametrics.com/blog/water-facts/>). Taking this into account, there comes several treatment methods such as flotation, filtering, sedimentation, agglomeration, oxidation, flocculation, chlorination, sorption through bio materials, sand filtration are used to treat the polluted water from industries as well as from sewage. Amongst all the above biosorption process was found to be the optimum method as it's a lot cheaper and effective method for treating. The treatment method involves the removal of congo red dye by using Hydrilla Verticillata Powder and the results were optimised through Box-Behnken design.

II. MATERIALS AND METHODS

Materials and methods engross the consequent steps

A. Reagents and Materials

All the components employed in this research were of analytical grade thus further need no purification. The primary source of Congo red dye solution was made ready by using distilled water. Analytical grade chemicals were used to avoid purification. 100mg of CR dye in 1litre will provide the stock and used as source of dye. By adding 0.1M HCL or 0.1 M NAOH the pH of solution can be made up to the mark.

B. Preparation of the Biosorbent

The fresh water algae *Hydrilla verticillata* was collected from River Godavari at Rajahmundry. A thorough wash with distilled water has been given to clear the dissolved impurities. The algae the dried in sunlight until it become crispy, then it is powdered to different sizes ranging from 53 micro meters to 152 micron meters

C. Studies on equilibrium biosorption process

A Prefixed volume of hydrilla powder is mixed well in a known volume of aqueous solution and is placed in an orbitrary shaker that has a predetermined time interval. Various factors such as agitation time, pH, dosage, initial concentration, temperature for the biosorption of CR can be identified by single step optimization and checked through Box-Benkhen design.

III.RESULTS AND DISCUSSION

A. Characterization of Fanwort Powder

- 1) *FTIR spectrum of untreated hydrilla powder:* A broad band at 573.85, 596.03, 617.25 and 663.54 cm^{-1} is due to the existence of C-Br stretch bands from alkyl halides. The broad absorption hits the highest point at around 758.06 cm^{-1} proves the incidence of Aromatic C-H bending group. The bands at 855.47, 876.68 and 937.44 cm^{-1} are due to the Al-O-H bending bonds. The bands at 1012.67 and 1024.25 cm^{-1} signifies the existence of C-F stretch bands from alkyl halides. The bands at 1037.75, 1053.18 and 1080.18 cm^{-1} are cause of the existence of C-F stretch bonds. The band at 1148.66 cm^{-1} proposes the incidence of Aromatic C-H bending bond. likewise the bands at 1211.35, 1233.53, 1242.21, 1319.37, 1339.62 and 1363.73 cm^{-1} are due to the occurrence of =C-H bending alkenes.

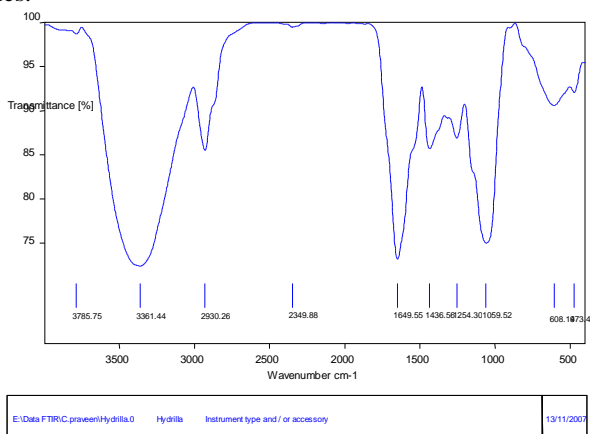


Fig 1. FTIR spectrum for untreated powder.

- 2) *FTIR Spectrum for Treated Powder:* A broad band at 617.25 cm^{-1} hints the attendance of C-Br stretch bands from alkyl halides. The band at 712.73 cm^{-1} is characteristic of Aromatic C-H bending bond. The band at 1495.86 cm^{-1} is due to the occurrence of Amine N-H stretch group. The bands at 3096.85, 3127.71, 3229.94, 3245.37, 3266.59 and 3287.81 cm^{-1} are the sign for the existence of Amine N-H stretching bonds. The bands at 3299.38, 3355.32, 3362.07 and 3372.68 cm^{-1} include -CH stretch bonds.

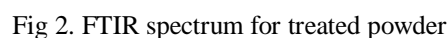


Fig 3 XRD pattern spectrum of untreated hydrilla powder

Fig 4 XRD pattern spectrum of treated hydrilla powder

- 5) *Scanning Electron Microscope*: SEM is a technique that is useful in studying both the natural sorbent morphology and its modification derived from sorbate interactions. SEM is an electron microscope that offers images of the sample surface by scanning it with a high energy beam of electrons. The electrons contacts with the atoms of the sample will produce signals that contain information about topography, morphology, and composition of the sample surface. The samples are to be electrically conductive, at least on their surface, for conventional SEM imaging. Nonconductive samples are coated with an ultra-thin layer of electrically conducting material. This coating averts the buildup of static electric charges on the sample surface throughout electron irradiation. Magnification of the imaging can be controlled over a range of up to 6 orders of magnitude from about $\times 25$ to 250,000 times. SEM-EDAX provides evidence for both the presence of dyes on the sorbent surface and micro precipitation of the dye. In this examination, possible mechanisms implicated in the sorption of the toxic elements in biomasses and variations due to the application of the amendments are investigated using SEM.
- 6) *Scanning Electron microscope (SEM)*: The SEM micrographs of hydrilla powder before and after biosorption are analyzed. The SEM images in figure show that the algae powder is less uneven and porous

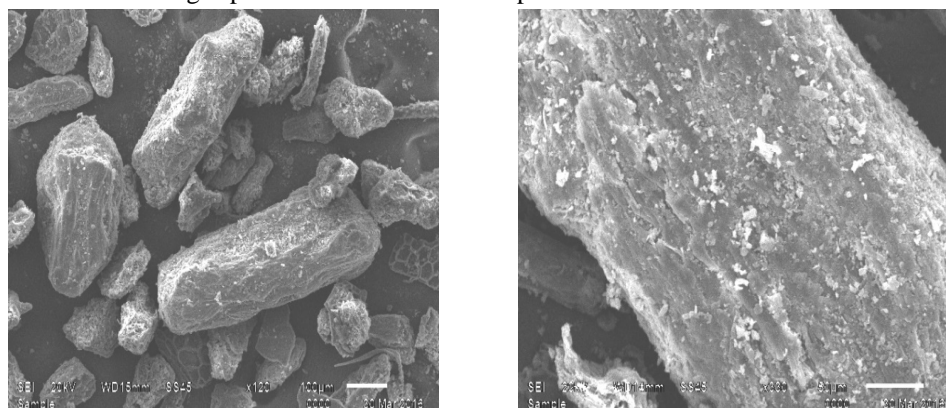


Fig 5. Untreated hydrilla powder

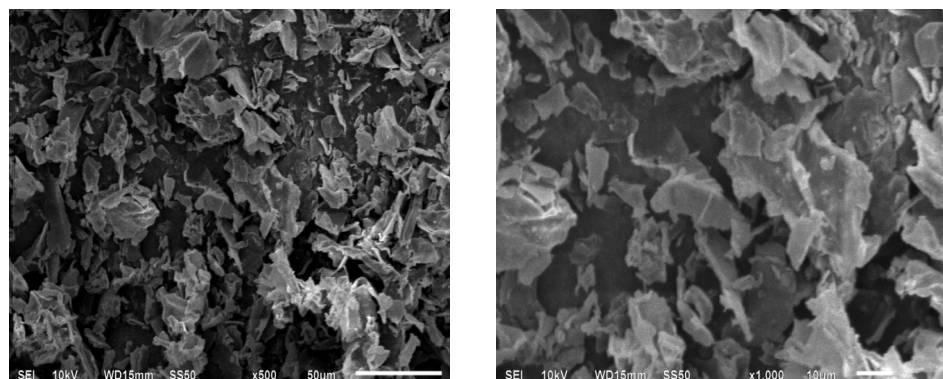


Fig 6. Treated hydrilla powder

B. Equilibrium studies on biosorption of Congo red

- 1) *Effect of agitation time*: The time required for dye concentration to reach a constant value during biosorption can be termed as the duration of equilibrium biosorption. The equilibrium agitation time is determined by plotting the % biosorption of CR dye against agitation time for the interaction time intervals between 1 to 180 min. For $53\mu\text{m}$ size of 0.5g/L biosorbent dosage, 26% of CR dye is biosorbed in the first 5min. The % biosorption is increased briskly up to 25min reaching 70%. Further than 25min, the % biosorption is constant which indicates the attainment of equilibrium conditions. The rate of biosorption is rapid in the initial stages because adequate surface area of the biosorbent is accessible for the biosorption of CR dye. As time increase, additional amount of CR dye gets biosorbed onto the surface of the biosorbent due to vanderwaal forces of attraction and resulted in decrease of available surface area. The maximum percentage of biosorption is achieved at 25minutes. Therefore, all other experiments are conducted at this optimum agitation time.

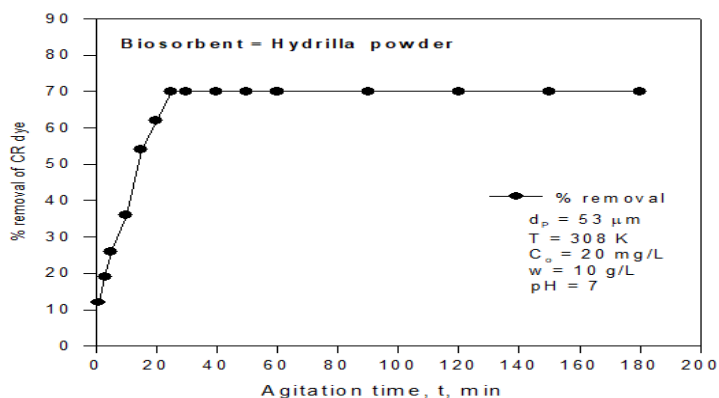


Fig 7 Effect of agitation time

- 2) *Effect of biosorbent size:* The variations in % biosorption of CR dye from the aqueous solution with biosorbent size are obtained. The biosorption percentage of CR dye is a function of biosorbent size. The percentage of biosorption is decreased from 70 % to 57 % as the biosorbent size decreases from $53\mu\text{m}$ to $152\mu\text{m}$. This phenomenon is projected, as the size of the particle decreases, surface area of the biosorbent increases; thereby the number of active and freely available sites on the biosorbent also increases.

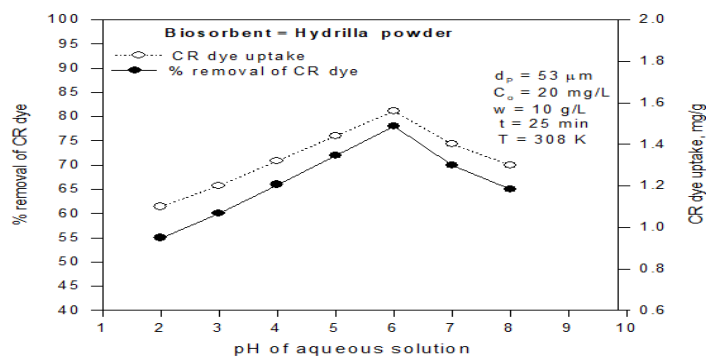


Fig 8 Effect of biosorbent size

- 3) *Effect of Ph:* pH controls biosorption by manipulating the surface change of the biosorbent, the degree of ionization and the species of biosorbate. In the present study, CR dye biosorption data are attained in the pH range of 2 to 8 of the aqueous solution ($C_0 = 20 \text{ mg/L}$) using 0.5g/L of $53\mu\text{m}$ size biosorbent. The biosorption percentage of CR dye is increased severely from 55% to 78% as pH is increased from 2 to 6 and beyond the pH value of 6 the increase and the margin are very low. Low pH lowers the biosorption due to competition with H^+ ions for suitable sites on the biosorbent surface. However, with increasing pH, this competition declines and BPB dye replace H^+ ions bound to the biosorbent.

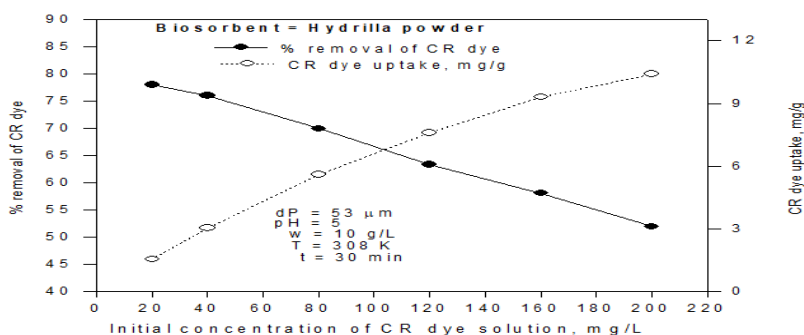


Fig 9 Effect of pH

- 4) *Effect of initial concentration of CR Dye:* The effects of initial concentration of CR dye in the aqueous solution on the biosorption percentage of CR dye are shown in the following diagram. The percentage biosorption of CR dye is decreased from 78% to 52% with an increase in C_0 from 20mg/L to 200mg/L. Such behavior can be helpful to the increase in the total amount of biosorbate to the constant number of available active sites on the biosorbent.

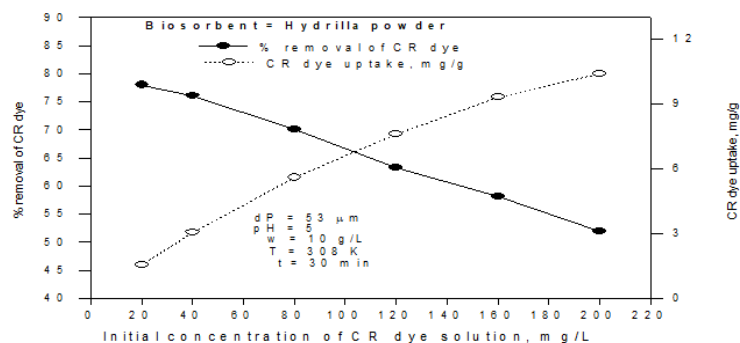


Fig 10 Effect of initial concentration

- 5) *Effect of biosorbent dosage:* The percentage biosorption of CR dye is drawn against biosorbent dosage for 53μm size biosorbent and is shown in the below mentioned diagram. The biosorption of CR dye improved from 78% to 92.5% with a raise in biosorbent dosage from 0.5 to 1.5g/L. Such behavior is obvious because with an increase in biosorbent dosage, the number of active sites available for CR dye biosorption would be more. The change in percentage biosorption of CR dye is marginal from 92.5% to 94.5% when 'w' is increased from 1.25 to 3g/L. Therefore all other experiments are conducted at 1.5g/L dosage.

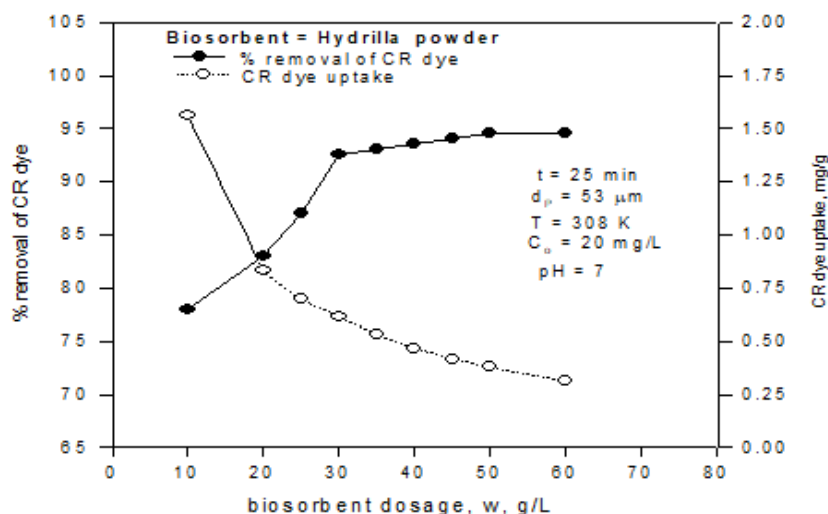


Fig 11 Effect of biosorbent dosage

- 6) *Effect of temperature:* The effect of temperature on the equilibrium dye uptake was significant. The effect of changes in the temperature on the CR dye uptake is portrayed in the following figure. High temperature favors the diffusion of dye molecules in the internal porous structure of surface. The biosorption of CR dye from 283 to 303K is increased from 87 to 92.5%, but after 303 the adsorption capacity is marginal.

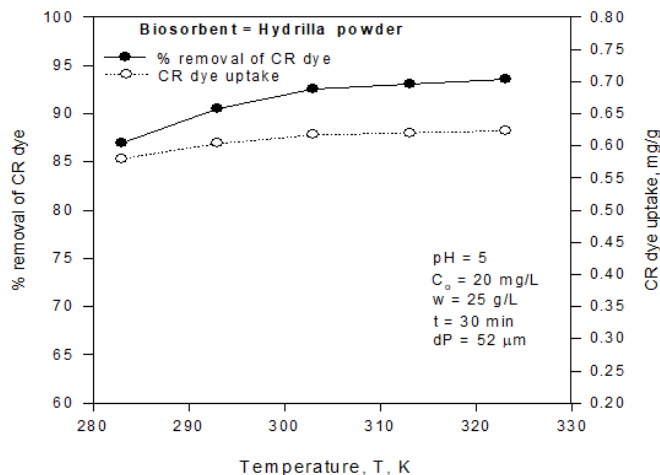


Fig 12 Effect of temperature

C. Kinetic studies

1) *Lagergren-first-order kinetic model* : The order of biosorbate – biosorbent interactions have been described using kinetic model. Traditionally, the first order model of Lagergren finds wide application. In the case of biosorption preceded by diffusion through a boundary, the kinetics in most cases follows the first order rate equation of Lagrangen:

$$(dq_t/dt) = K_{ad} (q_e - q_t)$$

Where, q_e and q_t are the amounts adsorbed at t , min and equilibrium time and K_{ad} is the rate constant of the pseudo first order biosorption. The above equation can be presented as

$$\int (dq_t / (q_e - q_t)) = \int K_{ad} dt$$

Applying the initial condition $q_t = 0$ at $t = 0$, we get

$$\log (q_e - q_t) = \log q_e - (K_{ad}/2.303) t$$

$$\log (q_e - q_t) = -0.0446t + 0.1618$$

Plot of $\log (q_e - q_t)$ versus ' t ' gives a straight line for first order kinetics, facilitating the computation of adsorption rate constant (K_{ad}).

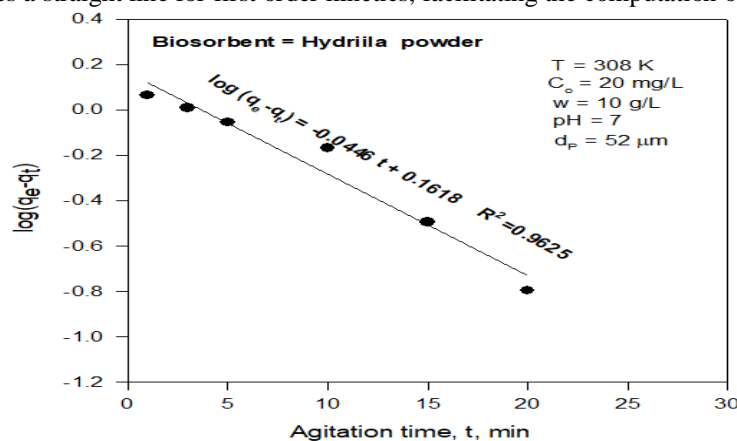


Fig 13 Lagergren-first-order kinetic model

2) *Pseudo second Order kinetics*: The below mentioned pseudo second order kinetic equation is applicable,

$$(dq_t/dt) = K (q_e - q_t)^2$$

Where, ' K ' is the second order rate constant.

The other form of the above equation is:

$$(dq_t / (q_e - q_t)^2) = K dt$$

$$1/x = Kx + C$$

$$C = 1/q_e \text{ at } t = 0 \text{ and } x = q_e$$

Substituting these values in above equation, we obtain:

$$1/(q_e - q_t) = Kt + (1/q_e)$$

Rearranging the terms, we get the linear form as:

$$(t/q_t) = (1/Kq_e^2) + (1/q_e) t.$$

$$(t/q_t) = 0.5674 t + 5.8236.$$

The pseudo second order model based on above equation, considers the rate-limiting step as the formation of chemisorptive bond involving sharing or exchange of electrons between the biosorbate and biosorbent. If the pseudo second order kinetics is applicable, the plot of (t/q_t) versus t gives a linear relationship that allows computation of q_e and K .

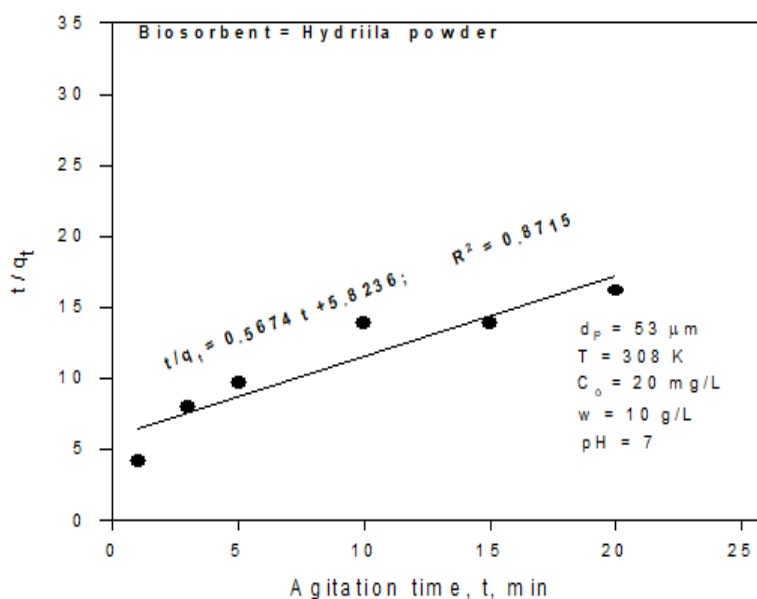


Fig 14 Pseudo second order kinetics

In the present study, the kinetics are investigated with 50 mL of aqueous solution ($C_0 = 20\text{mg/L}$) at 303K with the interaction time intervals of 1min to 180min. Lagrager plots of $\log (q_e - q_t)$ versus agitation time (t) for biosorption of CR dye the biosorbent size ($53 \mu\text{m}$) of *Hydrilla verticillata* powder in the interaction time intervals of 1 to 180min.

D. Isotherm models

1) *Langmuir model*: Irving Langmuir developed an isotherm named Langmuir isotherm. It is the most commonly used simple two-parameter equation. This simple isotherm is based on following assumptions: Biosorbates are chemically biosorbed at a fixed number of well-defined sites. Each site can hold only one biosorbate species. All sites are energetically equivalent. There are no interactions between the biosorbate species. The Langmuir relationship is hyperbolic and the equation is

$$q_e/q_m = bC_e / (1 + bC_e)$$

Equation can be rearranged as

$$(C_e/q_e) = 1/(bq_m) + C_e/q_m$$

From the plots between (C_e/q_e) and C_e , the slope $\{1/(bq_m)\}$ and the intercept $(1/b)$ are calculated. Further analysis of Langmuir equation is made on the basis of separation factor, (R_L) defined as $R_L = 1/(1 + bC_e)$. The following equation obtained 'n' with a good linearity (correlation coefficient, $R^2 \sim 0.9988$) indicating strong binding of CR dye to the surface of *hydrilla verticillata* powder.

$$C_e/q_e = 0.0699 C_e + 2.5574.$$

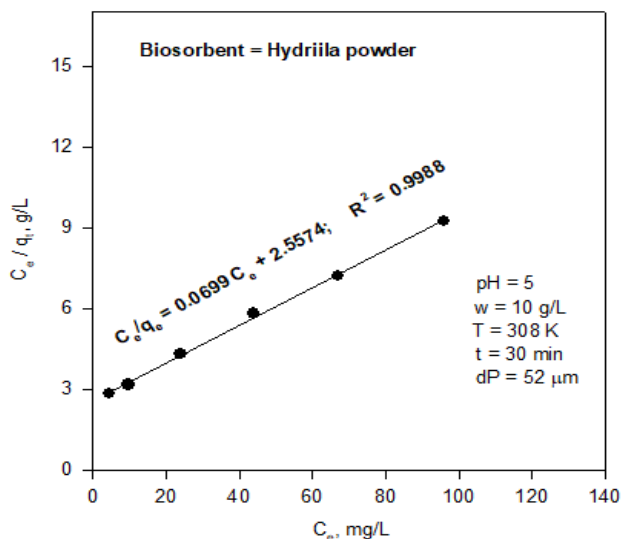


Fig 15 Langmuir isotherm

2) *Freundlich isotherm*: Freundlich presented an empirical biosorption isotherm equation that can be applied in case of low and intermediate concentration ranges. It is easier to handle mathematically in more complex calculations. The Freundlich isotherm is given by

$$q_e = K_f C_e^n$$

Where, K_f (mg) represents the biosorption capacity when dye equilibrium concentration and n represents the degree of dependence of biosorption with equilibrium concentration Taking logarithms on both sides, we get

$$\ln q_e = \ln K_f + n \ln C_e$$

Freundlich isotherm is drawn between $\ln q_e = 0.6146 \ln C_e - 0.3484$; $\ln C_e$ and $\ln q_e$ The resulting equation has a correlation coefficient of 0.9820. The 'n' value in the above equations satisfies the condition of $0 < n < 1$ indicating favorable biosorption.

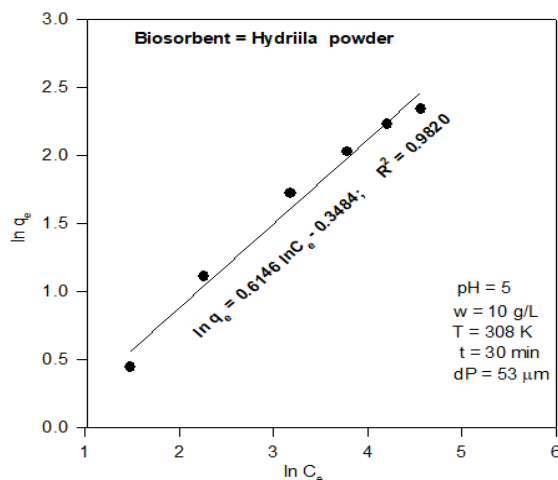


Fig 15 Freundlich isotherm

3) *Temkin isotherm*:

Temkin and Pyzhev isotherm equation describes the behaviour of many biosorption systems on the heterogeneous surface and it is based on the following equation:

$$q_e = RT \ln(A_T C_e) / b_T$$

The linear form of Temkin isotherm can be expressed as

$$q_e = (RT / b_T) \ln(A_T) + (RT / b_T) \ln(C_e)$$

The equation obtained for BPB dye biosorption is

$$q_e = 2.9290 \ln C_e - 3.2568 \text{ with a correlation coefficient } 0.9880.$$

The best fit model is determined based on the linear regression correlation coefficient (R).

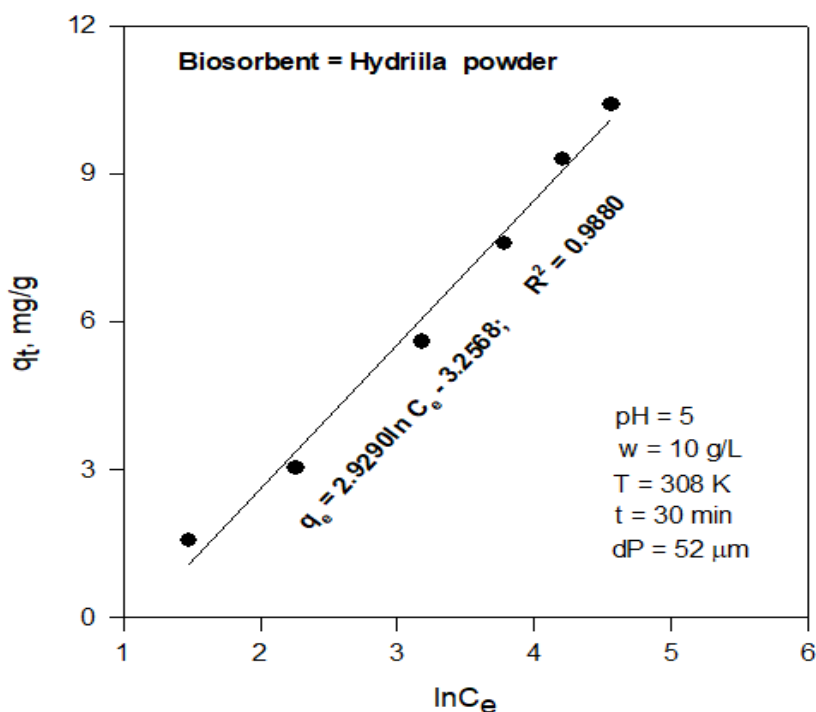


Fig 15 Temkin isotherm

TABLE I
ISOTHERMS

Langmuir isotherm	Freundlich isotherm	Temkin isotherm
$q_m = 14.28571 \text{ mg/g}$	$K_f = 0.7051 \text{ mg/g}$	$A_T = 0.3289 \text{ L/mg}$
$K_L = 0.02737$	$n = 0.6146$	$b_T = 860.069$
$R^2 = 0.9988$	$R^2 = 0.9820$	$R^2 = 0.9880$

E. Thermodynamics

1) *Vanthoff's plot*: Biosorption is temperature dependant. In general, the temperature dependence is linked with three thermodynamic parameters namely change in enthalpy of biosorption (ΔH), change in entropy of biosorption (ΔS) and change in Gibbs free energy (ΔG). The Vant Hoff's equation is

$$\log (q_e / C_e) = \Delta H / (2.303 RT) + (\Delta S / 2.303 R)$$

$$\log (q_e / C_e) = -0.7485 (1 / T) + 2.0342.$$

Where, (q_e / C_e) is called the biosorption affinity.

If the value of ΔS is less than zero, it shows that the process is highly reversible. If ΔS is more than or equal to zero, it indicates the reversibility of process. The negative value for ΔG indicates the spontaneity of biosorption, whereas the positive value resembles the non spontaneity of sorption. Experiments are carried out to understand the biosorption behavior by varying the temperature from 283 to 323K. The plots signify the effect of temperature on biosorption of CR dye for different initial CR dye concentrations. The Vant Hoff's plots for the biosorption data obtained at various initial concentrations of the CR dye.

The values are $\Delta G = -11787.3$, $\Delta H = 14.3316$ and $\Delta S = 38.949$

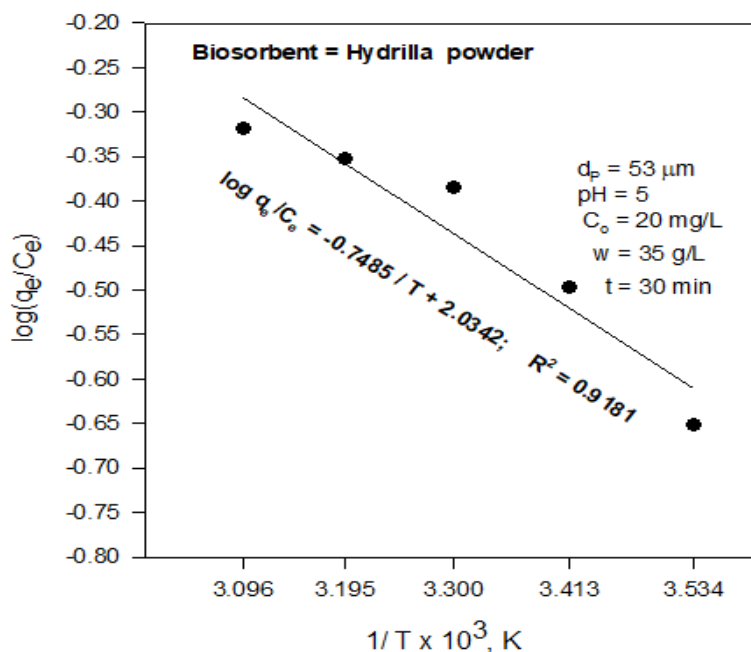


Fig 16. Vanthoff's plot

F. Optimization using Response Surface Methodology (RSM)

Optimization of the selected parameters using BBD:

The parameters that have greater influence over the response are to be identified so as to find the optimum condition for the biosorption of CR dye. The quadratic model is used in the present study, to relate four independent variables and percentage biosorption of CR dye. The regression equation for its % biosorption of CR dye is function of pH (X_1), C_o (X_3), w (X_2) and T (X_4).

Table ii

The variations in the corresponding coded values of four parameters and response

Variable	Name	Range and levels		
		-1	0	+1
X1	pH of aqueous solution	5	6	7
X2	Initial concentration, C_o , mg/L	10	20	30
X3	Biosorbent dosage, w , g/L	1	1.5	2
X4	Temperature, K	293	303	313

The following equation represents multiple regression analysis of the experimental data for the biosorption of Congo Red:

$$Y = -1364.39 + 37.32 X_1 + 2.39 X_2 + 4.23 X_3 + 8.49 X_4 - 4.53 X_1^2 - 0.06 X_2^2 - 0.07 X_3^2 - 0.01 X_4^2 + 0.00 X_1 X_2 - 0.01 X_1 X_3 - 0.00 X_1 X_4 + 0.00 X_2 X_3 - 0.00 X_2 X_4 + 0.00 X_3 X_4 \text{ ---- (10)}$$

Run No.	X ₁ ,pH	X ₂ , C ₀	X ₃ , w	X ₄ , T	% removal of CR dye	
					Experimental	Predicted
1	5	10	1.5	303	93.2	87.61
2	7	10	1.5	303	91.2	87.07
3	5	30	1.5	303	90.8	88.59
4	7	30	1.5	303	90.8	88.06
5	6	20	1.25	293	87.6	80.97
6	6	20	1.75	293	90.6	84.87
7	6	20	1.25	313	88.9	82.78
8	6	20	1.75	313	91.6	86.68
9	6	20	1.5	303	90.6	90.61
10	5	20	1.5	293	89.8	86.89
11	7	20	1.5	293	89.2	86.36
12	5	20	1.5	313	93.2	88.70
13	7	20	1.5	313	93	88.17
14	6	10	1.25	303	92.1	81.68
15	6	30	1.25	303	91.6	82.67
16	6	10	1.75	303	93.1	85.58
17	6	30	1.75	303	92.1	82.67
18	6	20	1.5	303	91	90.61
19	5	20	1.25	303	88.3	82.57
20	7	20	1.25	303	90.1	82.04
21	5	20	1.75	303	92.1	86.47
22	7	20	1.75	303	91.6	85.94
23	6	10	1.5	293	89.6	86.00
24	6	30	1.5	293	88.6	86.99
25	6	10	1.5	313	90.2	87.81
26	6	30	1.5	313	91.2	88.80
27	6	20	1.5	303	93.2	90.61
28	6	20	1.5	303	93.2	90.61
29	6	20	1.5	303	93.2	90.61
30	6	20	1.5	303	93.2	90.61

Table III
Annova for cr removal for total quadratic model

Source of variation	SS	df	Mean square(MS)	F-value	P> F
Model	260.6469	14	18.6176	103431	0.00000
Error	0.0028	15	0.00018		
Total	260.6497				

Term	Regression	Standard error	T(15)	p
Mean/Interc.	-1654.39	4.090951	-404.40	0.00
(1)pH (L)	15.63	0.053132	294.23	0.00
pH (Q)	-1.32	0.004419	-299.84	0.00
(2)Initial Concentration(L)	0.63	0.001803	350.08	0.00
Initial Concentration(Q)	-0.01	0.000044	-327.16	0.00
(3)Dosage (L)	64.29	0.052913	1215.02	0.00
Dosage (Q)	-20.13	0.017790	-1131.57	0.00
(4)Temperature(L)	10.74	0.026781	401.07	0.00
Temperature(Q)	-0.02	0.000044	-397.72	0.00

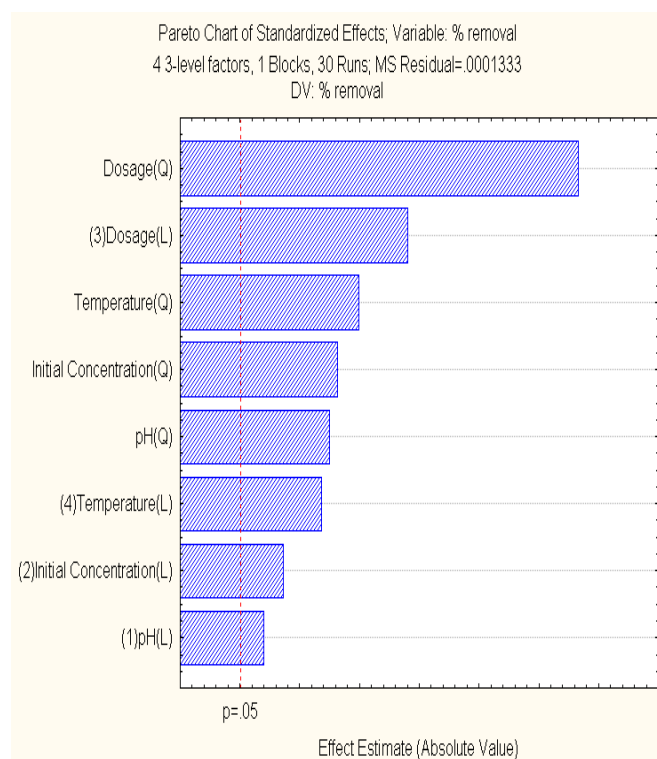


Fig 17 Pareto chart

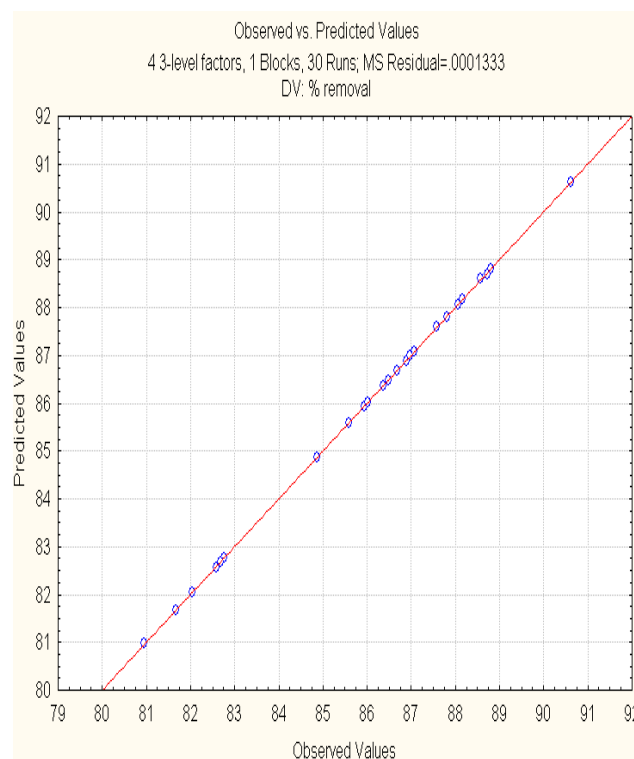


Fig 18 normal probability %biosorption of CR

G. Interaction effects of biosorption variables

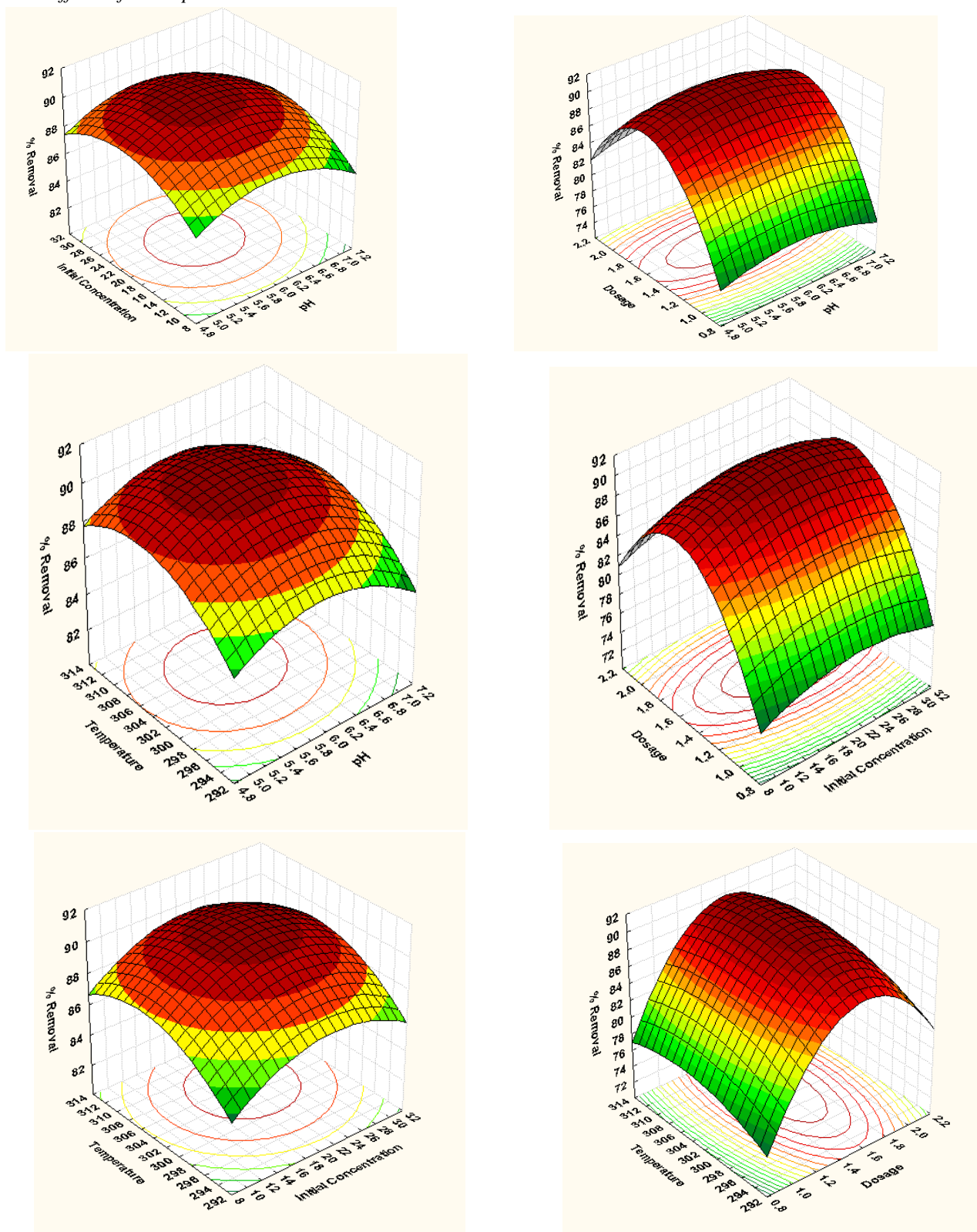


Fig 19. Surface contour plot for the effects of dosage and initial concentration on %, pH on %, Temperature on % biosorption of congo red.

TABLE IV
Comparison between optimum values from BBD and experimentation CR with hydrilla

Variable	BBD	Experimental
pH of aqueous solution	5.8994	6
Initial CR concentration, mg/L	21.6952	20
Biosorbent dosage, w, g/L	1.5969	1.5
Temperature, K	303.5747	303
% biosorption	90.97394	92.5

IV.CONCLUSIONS

Comparison between optimum values from BBD and experimentation CR with Hydrilla:

Hydrilla powder performance on CR dye:

The equilibrium agitation time for CR dye biosorption is 25 minutes. The optimum dosage for biosorption is 30 g/L. Maximum extent of biosorption is noted at pH = 6. From the predicted values of RSM results, maximum biosorption of CR dye (90.97394 %) is observed when the processing parameters are set as pH = 5.8994, w = 1.5969 g, C₀ = 21.6952mg/L and T = 305.5747 K. The investigation also reveals the endothermic nature of biosorption as ΔH is positive (14.3316 J/mole) spontaneity of the biosorption as ΔG is negative (–11787.3 J/mole) and irreversible nature of biosorption as ΔS is positive (38.949)

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