

# DECOLORIZATION POTENTIAL OF IMMOBILIZED *PSEUDOMONAS PUTIDA* MTCC 1194 WITH LOW COST ADSORBENT FOR REACTIVE DYE

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## Abstract

A simultaneous adsorption and biodegradation method for removal of reactive blue-4 is reported here. The mustard stalk activated carbon (MSAC) using *Pseudomonas putida* MTCC1194 was applied to decolorize reactive blue-4. Batch studies were performed to evaluate the influences of various parameters; initial pH, adsorbent dose, temperature and initial concentration, on removal of reactive blue-4. Optimum conditions for reactive blue -4 removals were found to be pH 7, adsorbent dose 10g/l, temperature 32°C at equilibrium time 360 min for 150 mg/l of dye concentration. Experimental data were analyzed by pseudo-first order, pseudo-second order kinetics and intra-particle diffusion model. Equilibrium isotherms for the adsorption of reactive blue 4 onto MSAC were analyzed by Freundlich, Langmuir, Temkin and Dubinin–Radushkevich (D-R). The results show that experimental data follow pseudo-second order kinetics and intra-particle diffusion model. Out of four isotherms, Langmuir was found to be best fit with experimental data ( $R^2 > 0.97$ ), with 70.2% removal of reactive blue 4.

**Keywords:** activated carbon mustard stalk, reactive blue 4, simultaneous adsorption and biodegradation (SAB), *Pseudomonas putida*

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Symbol	Description	Unit
1/ n	Heterogeneity factor, dimensionless	
B	Dubinin–Radushkevich model constant	(mol <sup>2</sup> k J <sup>-2</sup> )
B <sub>1</sub>	Heat of adsorption	
C <sub>0</sub>	Initial concentration of adsorbate in solution	(mg l <sup>-1</sup> )
C <sub>e</sub>	Equilibrium liquid phase concentration	(mg l <sup>-1</sup> )
C <sub>t</sub>	Concentration at time t	(mg l <sup>-1</sup> )
E	Mean energy of sorption	(kJ mol <sup>-1</sup> )
h	Initial sorption rate	(mg g <sup>-1</sup> min <sup>-1</sup> )
I	Boundary layer	
k	Rate constant of pseudo second- order sorption	(gmg <sup>-1</sup> min <sup>-1</sup> )
K <sub>f</sub>	Freundlich constant	((mg g <sup>-1</sup> ) (mg l <sup>-1</sup> ) <sup>-1/n</sup> )
k <sub>i</sub>	Rate constant of pseudo first order sorption	(min <sup>-1</sup> )
k <sub>id1</sub>	Intra-particle diffusion rate constant. at the first step	( mg g <sup>-1</sup> min <sup>1/2</sup> )
k <sub>id2</sub>	Intra-particle rate constant transport at second step	( mg g <sup>-1</sup> min <sup>1/2</sup> )
K <sub>L</sub>	Langmuir adsorption constant	(l mg <sup>-1</sup> )
K <sub>T</sub>	Equilibrium binding constant	(l mg <sup>-1</sup> )
q <sub>e</sub>	Sorption capacities at equilibrium	(mg g <sup>-1</sup> )
Q <sub>m</sub>	Theoretical maximum adsorption capacity	(mg g <sup>-1</sup> )
Q <sub>s</sub>	Theoretical monolayer saturation capacity	(mg g <sup>-1</sup> )
q <sub>t</sub>	Sorption capacities at time t	(mg g <sup>-1</sup> )
R	Universal gas constant	(8.314 J K <sup>-1</sup> mol)
R <sup>2</sup>	Correlation coefficient	
R <sub>L</sub>	Separation factor, dimensionless	
T	Temperature	(°C)
t	Time	Min.
α	Initial sorption rate	(mg g <sup>-1</sup> min <sup>-1</sup> )
β	Desorption constant	(g mg <sup>-1</sup> )
ε	Polanyi potential	

## ABBREVIATION:

## 1. INTRODUCTION

Dye containing wastewaters are very difficult to treat due to their high COD, BOD, suspended solids and toxic compound contents and the aesthetic issues raised by easily recognized colors. Due to large-scale production and extensive application, synthetic dyes can cause considerable environmental pollution and are serious health-risk factors [23]. The improper disposal of dyes leads to the reduction in photosynthetic activity which adversely affecting the aquatic life which cause a potential health hazard. Textile industry workers exposed to reactive dyes suffers with, changes in their immunoglobulin levels, allergic dermatitis and respiratory diseases [16].

To eliminate dyes from aqueous colored effluents, several physical, biological and chemical techniques have been used [17]. Among various methods adsorption has been found to be useful techniques as low-cost, ecofriendly for treating wastewater. The low-cost adsorbent materials obtained from agricultural wastes that have been converted to activated carbon for use in dye adsorption as reported by various researchers, i.e. olive kernels [25], *Euphorbia rigida* [4], oil palm trunk fiber [5] and jute fiber [18] for methylene blue removal, coconut flower for reactive red adsorption [19], bamboo dust, coconut shell, groundnut shell, rice husk and straw for removal of Congo red and silk cotton hull for reactive blue removal [20] etc.

The simultaneous adsorption-biological treatments is effective for treating dyes and organic toxic pollutants of industrial wastewaters due to minimum sludge production and **improve** water quality in the most economical way [3,15].

Reactive dyes often used for cellulosic fabrics have environmental implications [8]. Reactive blue 4 (RB-4) an anthraquinone reactive dye, highly water soluble and non-degradable, adsorb poorly to biological solids and remain in the discharged effluents [2,17]. Therefore our aim to eliminate reactive blue-4 by simultaneous adsorption and biodegradation by mustard stalk activated carbon (MSAC) immobilized with *Pseudomonas putida* MTCC 1194. The process parameters like adsorbent dose, initial concentration, contact time, temperature and pH have been optimized to find out percent removal of reactive blue-4. The kinetic, intra-particle diffusion model and adsorption isotherms, used to evaluate the experimental data.

## 2. MATERIALS AND METHODS

Activated carbon prepared from the mustard stalk by the procedure mention by Ullhyan et al. 2014 [22]. The physico-chemical characteristics of MSAC were determined using standard procedures by ASTM. The surface area of activated carbon is a key factor because, like other physical-chemical characteristics, it may strongly affect the adsorption capacity of activated carbon. Nitrogen adsorption experiments at 77.15 K were conducted to determine the specific surface area of the mustard stalk activated carbon using an (ASAP 2010 Micrometrics) surface area analyzer. In order to investigate the surface morphology of MSAC, scanning

electron microscope (Model SEM-501, Phillips, Holland) was used.

The commercial reactive blue-4 (abbreviation: RB-4, CI number: 61205; molecular formula:  $C_{23}H_{12}C_{12}N_6Na_2O_8S_2$ ) was purchased from Sigma Aldrich (Germany). An accurately weighed quantity of the dye was dissolved in double-distilled water to prepare a stock solution (1000 mg/l). The desired concentration range 50–200 mg/l was obtained by successive dilutions with double-distilled water.

### 2.1 Microorganism and culture conditions

*Pseudomonas putida* MTCC 1194 was obtained from Institute of Microbial Technology, Chandigarh, India. Nutrient agar medium and basal salt medium were used for microbial growth. Nutrient agar medium contained 1 g beef extract, 2 g yeast extract, 5 g peptone, 5 g NaCl and 15 g agar in one liter distilled water. The composition of the basal salt medium (BSM) used in this experiment as the growth medium contained 1.5 g/l  $K_2HPO_4$ , 0.5 g/l  $KH_2PO_4$ , 0.5 g/l  $(NH_4)_3PO_4$ , 0.5 g/l NaCl, 3 g/l  $Na_2SO_4$ , 2 g/l Yeast extract, 0.5 g/l Glucose, 0.002 g/l  $FeSO_4$  and 0.002 g/l  $CaCl_2$ .

#### 2.1.1 Experimental procedure

##### Acclimatization of culture

The acclimatization of *Pseudomonas putida* (MTCC1194) in dye environment was performed as follows: the revived culture was first grown in basal salts medium (BSM) with glucose in a 250 ml cotton-plugged conical flask for 48 hours, significant bacterial growth was observed by turning culture into milky form. Acclimatization of culture was performed in batch mode in orbital shaker at 32°C and 180 RPM in 250 ml cotton-plugged conical flasks containing basal salt medium, bacterial inoculums and stock solution of dye concentration, ranging from 10 mg/l to 250 mg/l, with increment of 10 mg/l in a series till the cumulative concentration in the growth medium reached 100 mg/l. It was kept aside initially, until the growth of *Pseudomonas putida* was inhibited [10].

For batch study, experiments were conducted in 500 ml cotton-plugged flasks containing BSM with 20 ml inoculums of acclimatized *Pseudomonas putida* with dye having biomass concentration 18.72 mg/l, a dye aliquot of 40 ml of stock solution of dye and definite amount of adsorbent dose. The reaction mixture was agitated in orbital shaker at constant speed of 180 RPM. Initial concentration of reactive blue-4 was varied between 50 to 200 mg /l, having adsorbent dose varied from 2 to 12g. The pH range 2 to 9 and temperatures vary from 25 °C to 35 °C, respectively. Samples were collected at definite intervals of time. All the collected samples were centrifuged at 10,000 RPM for 15 min. The supernatant was separated and analyzed spectrophotometrically at 595nm using a double beam UV/VIS spectrophotometer (Perkin-Elmer 135). Various parameters, i.e. initial concentrations of dye, adsorbent dose, pH, contact time and temperature were studied to determine kinetic models and adsorption

isotherm. The percentage removal of dye (eq. 1) and equilibrium adsorption uptake,  $q_e$  (mg/g), (eq.2) were calculated using the following relationships:

$$\text{Dye Removal (\%)} = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

$$\text{Amount adsorbed } (q_e) = \frac{(C_0 - C_e)v}{w} \quad (2)$$

Where  $C_0$  (mg/l) is the initial adsorbate concentration,  $C_e$  is equilibrium concentration and  $C_t$  is a concentration at time  $t$ ,  $v$  the volume of the solution (l) and  $w$  is the mass of the adsorbent (g). Statistical software Data fit 9.0 has been used for this study which utilizes the Levenberg-Marquardt method with double precision to perform nonlinear regression.

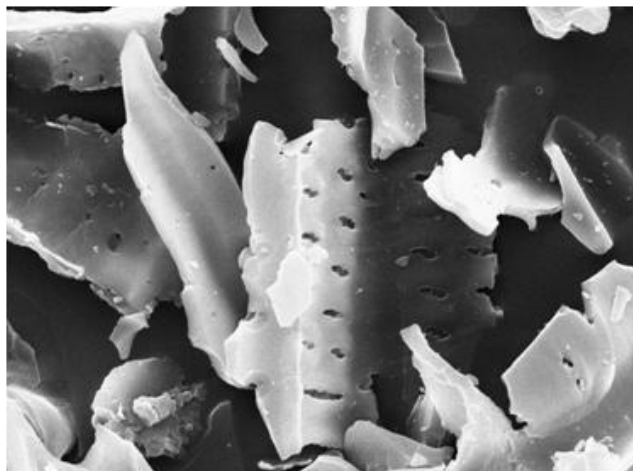
### 3. RESULT AND DISCUSSION

#### 3.1 Characterization of adsorbent

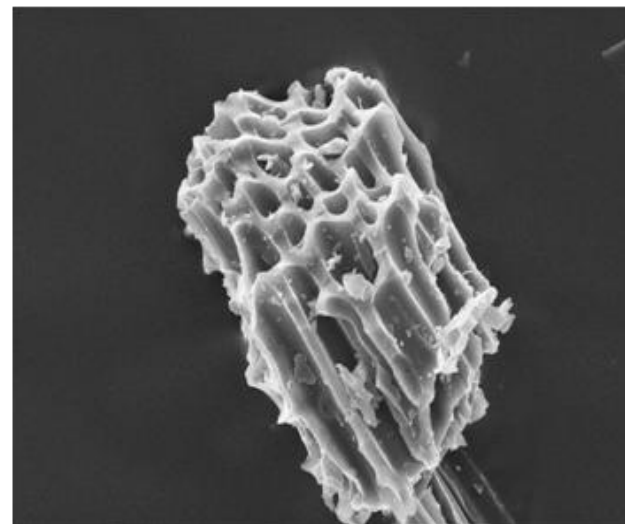
Physical-chemical characteristics of mustard stalk activated carbon are presented in Table 1 and show that it contains carbon, nitrogen, sulfur, hydrogen, etc. Characterization of MSAC show BET surface area  $129 \text{ m}^2/\text{g}$ , BJH adsorption average pore diameter  $1.505 \text{ \AA}$  and BJH cumulative pore volume  $13.56 \text{ cc/g}$ , which are good for adsorption process. Scanning electron microscopy (SEM) was used to characterize the morphology of MSAC. The SEM micrographs of fresh mustard stalk fig. 1. A, shows plain surface, but after chemical activation of mustard stalk shown in fig.1. B, having a linear type of fibers with holes and skeletal like structure in it. The well-developed pores had led to large surface area and porous structure which confirms that there is a good possibility for the adsorbate to be trapped and get adsorbed into these pores. Figure 1.C shows that holes/cavities of MSAC are filled after simultaneous adsorption and biodegradation of reactive blue-4. The characterization results, showed that low cost activated carbon prepared from mustard stalk (MSAC) using chemical activation method ( $\text{H}_2\text{SO}_4$ ) have a good indication of suitability of the mustard stalk as an adsorbent.

**Table.1.** Characteristics of activated carbon prepared from mustard stalk (MSAC)

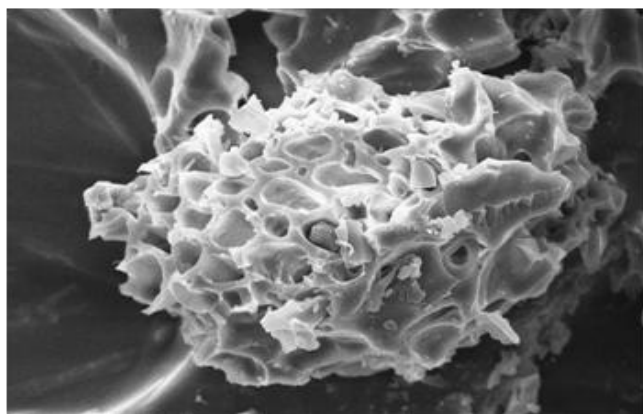
Properties	Value
Ash content (%)	6.5
Fixed carbon (%)	0.95
Bulk density ( $\text{g}/\text{cm}^3$ )	0.37
Volatile matter (%)	19
Moisture (%)	6
Particle size (mm)	2-4
Iodine number ( $\text{mg g}^{-1}$ )	730
Methylene blue number ( $\text{mg g}^{-1}$ )	290
BET surface area ( $\text{m}^2/\text{g}$ )	129
BJH cumulative pore volume ( $\text{cc/g}$ )	1.505
BJH adsorption average pore diameter ( $\text{\AA}$ )	13.56
pH	6.5
C %	74.6
N %	3.13
H %	1.4
O %	1.6
S %	1.0



**Fig-1:A** SEM micrographs of fresh mustard stalk at magnification 600 x



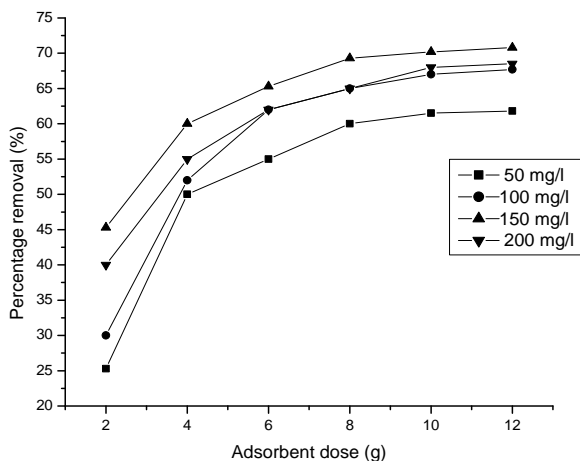
**Fig-1:B** SEM micrographs of mustard stalk after activation at magnification 600 x



**Fig-1:**C SEM micrographs after SAB of Reactive blue-4 onto MSAC at magnification 600 x

### 3.2 Effect of adsorbent dose

Figure 2 reveals that removal of dye increase with increase at adsorbent dose 10 g/l, due to greater surface area and availability of more adsorption sites, but after adsorbent dose larger than 12 g/l removal of dye almost unchanged. Beyond adsorbent dose of 10 g/l, percent removal of dye becomes almost constant indicating that the surface dye concentration and the solution dye concentration tend to reach equilibrium. So it does not make a significant effect on further increases in adsorbent dose. Hence, 10 g/l of adsorbent dose has been used as optimum dose for this study. Similar results were obtained [21] for adsorption of dyes on low cost activated carbon.

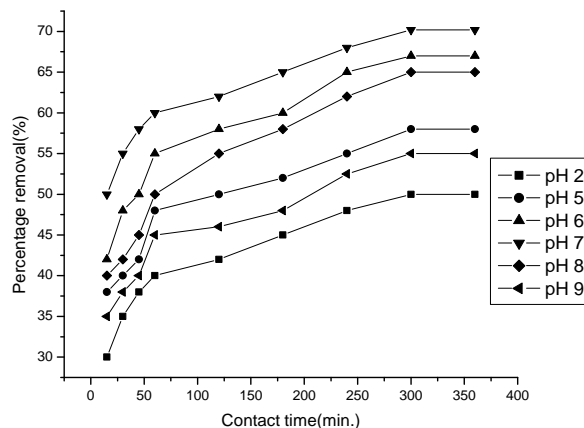


**Fig-2:** Effect of adsorbent dose of removal of Reactive blue-4 onto MSAC. At pH 7, temp. 32°C.

### 3.3 Effect of pH

Figure 3 shows that on either side of pH 7, percent decolourisation decreased as the pH increased to acidic or alkaline range. The maximum removal of dye occurs at optimal pH 7 and percentage removal capacity increases when the pH is increased from 2 to 7, beyond pH 7 the percentage removal of reactive blue-4 slightly decreased. The large reduction in dye adsorption at higher basic conditions can be attributed to electrostatic repulsion

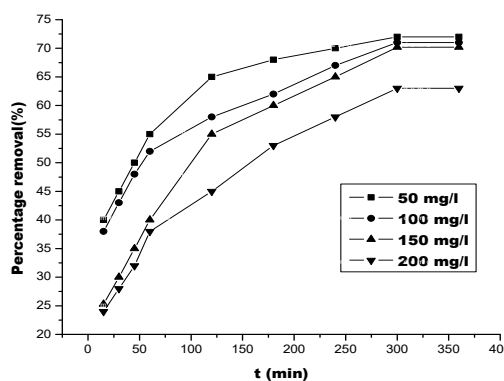
between the negatively charged activated carbon and the deprotonated dye molecules. [13,14].



**Fig-3:** Effect of pH on removal of Reactive blue-4 onto MSAC. At initial conc.150 mg/l, adsorbent dose 10 g/l, temp. 32°C

### 3.4 Effect of initial dye concentration

Figure 4 the effect of initial concentration (50–200 mg/l) of reactive blue-4 onto MSAC show rapid adsorption in 240 min i.e. 70.2 % removal and thereafter the adsorption rate decreased gradually and the adsorption reached equilibrium in about 360 min. SAB curves are single, smooth and continuous leading to saturation and indicated the possible monolayer coverage on the surface of the adsorbent by the dye molecules [12,24]. The effect of initial dye concentrations observed in this study suggests that the increase in the initial concentration enhances the interaction between dye and MSAC [6].



**Fig-4:** Effect of initial concentration on removal of Reactive blue-4 onto MSAC. At adsorbent dose 10 g/l, pH 7, temp. 32°C

### 3.5 Effect Of Temperature

Figure 5 shows that to rise in temperature from 28°C to 35°C removal of reactive blue-4 increases. This is due to an increase in temperature, the mobility of the reactive blue-4 ions increases and the retarding forces acting on the diffusing ions decrease, thereby increasing the sorptive

capacity of adsorbent. Therefore, the increase in sorption capacity with an increase in temperature may be attributed to chemisorptions [9]. Beyond 35°C bacteria stops degradation due to slowdown of metabolic activity, then becomes dead, which hindered its biodegradation capability. Hence, for this study 32°C temperature was selected as the optimum temperature.

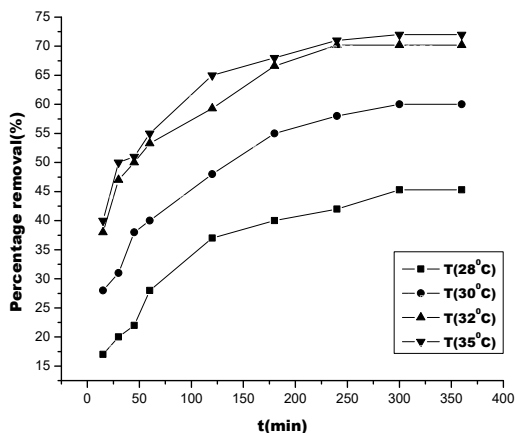


Fig-5: Effect of temperature on Reactive blue-4 removal onto MSAC. At initial conc. 150 mg l<sup>-1</sup>, adsorbent dose 10 g l<sup>-1</sup>, pH 7

#### 4. KINETIC AND ISOTHERMS STUDY

To investigate the mechanism of reactive blue-4 adsorption-biodegradation, kinetic models, that is pseudo first order, pseudo second order, and intra particle diffusion was considered to interpret the experimental data.

The pseudo-first-order model was described by Lagergren (eq. 3).

$$\log(q_e - q_t) = \log(q_e) - \frac{k_i}{2.303} t \quad (3)$$

Where q<sub>e</sub> and q<sub>t</sub> refer to the amount of dye adsorbed (mg/g) at equilibrium and at any time, t (min), respectively, and k<sub>i</sub> are the equilibrium rate constant of pseudo-first-order adsorption (min<sup>-1</sup>). The values of log (q<sub>e</sub> - q) were linearly correlated with it. As shown in Table 2, pseudo-first order equation did not fit well for most of the range of concentrations under study with lower correlation coefficient (R<sup>2</sup> 0.562). For this reason, the Lagergren expression cannot be applied to the entire process of adsorption-biodegradation of reactive blue-4 onto MSAC.

The pseudo second order chemisorption kinetic rate equation is expressed as:

$$\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{1}{q_e} t \quad (4)$$

Where, q<sub>e</sub> and q<sub>t</sub> are the sorption capacities at equilibrium and at time t, respectively, and k is the rate constant of pseudo second order sorption. The initial sorption rate h, as q<sub>t</sub>/t → 0, can be defined as:

$$h = kq_e^2 \quad (5)$$

Hence, eq. (5) could be written as:

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t \quad (6)$$

The slope and intercept of plot of t/q vs. t were used to calculate the second-order rate constant (Fig.6). The values of the equilibrium rate constant are presented in Table 2. The correlation coefficients of all examined data were found very high (R<sup>2</sup> ≥ 0.998). This confirms that the sorption of reactive blue-4 onto MSAC follows the pseudo-second order kinetic model. The results indicated that the rate-limiting step may be chemical sorption.

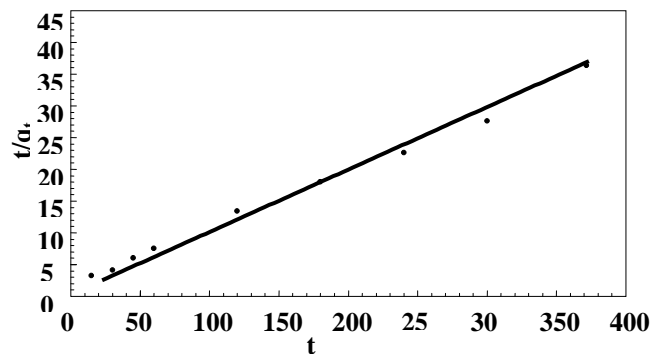


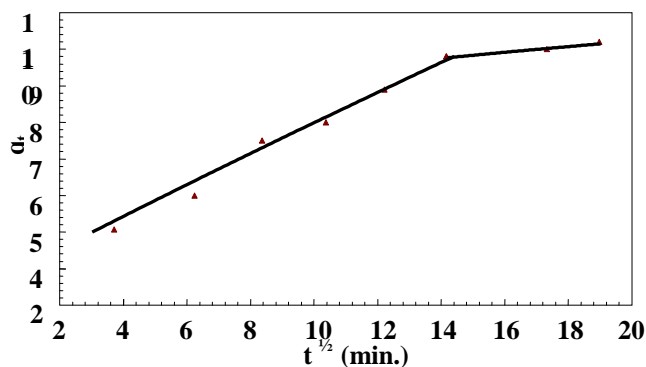
Fig-6: Pseudo-second order kinetics for Reactive blue-4 removal onto MSAC. At initial conc. 150 mg/l, adsorbent dose 10 g/l, pH 7, temp. 32°C

#### 4.1 Intra-particle diffusion

Pseudo-first order and second-order model could not identify the diffusion mechanism; the kinetic results were further analyzed by the intra-particle diffusion model by Weber and Morris to elucidate the diffusion mechanism. The amount of reactive blue-4 adsorbed (q<sub>t</sub>) at a time (t) was plotted against the square root of contact time (t<sup>0.5</sup>) according to eq. (7)

$$q_t = K_{id} t^{1/2} + I \quad (7)$$

Where k<sub>id</sub> is the intra-particle diffusion rate constant. The values of I give an idea about the thickness of the boundary layer, i.e., the larger the intercept, the greater is the boundary layer effect. Figure 7, a plot of q<sub>t</sub> versus t<sup>1/2</sup> is presented intra-particle diffusion of reactive blue-4 onto MSAC. The present study indicates that the initial portion of reactive blue-4 adsorption by MSAC may be governed by the initial intra-particle transport of dye, controlled by surface diffusion process and the later part controlled by pore diffusion. The diffusion rate was found high in the initial stages (k<sub>id,1</sub> 4.45 mg/g/min.) and decreased with the passage of time (k<sub>id,2</sub> 0.014 mg/g/min.). The values of rate parameters are given in Table 2 indicate that intra-particle diffusion step could be a rate-controlling step [11].



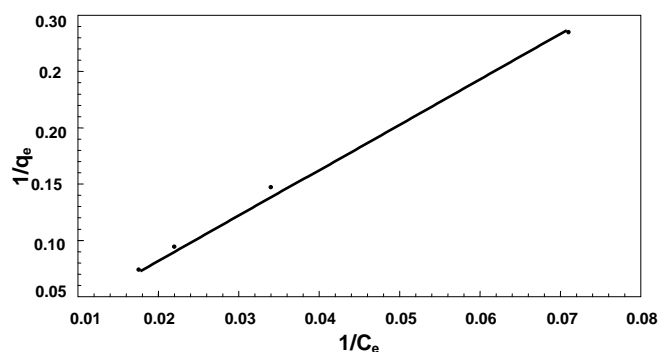
**Fig-7:** Weber Morris intra-particle diffusion plot for Reactive blue-4 removal onto MSAC. At initial conc.150mg/l, adsorbent dose 10 g/l, pH 7, temp. 32°C

### 4.2 Langmuir isotherm

The Langmuir equation is represented in the linear form as follows:

$$\frac{C_e}{q_e} = \frac{1}{K_L Q_m} + \frac{C_e}{Q_m} \tag{8}$$

Figure 8 shows the Langmuir (1/ C<sub>e</sub> vs. 1/ q<sub>e</sub>) plot of reactive blue-4 onto MSAC. The isotherm of activated carbon is found to be linear over the whole concentration range. Q<sub>m</sub> is the theoretical maximum adsorption capacity (25.8 mg/g) and the correlation coefficients are extremely high, R<sup>2</sup> 0.999 as shown in Table 2. This confirms, Langmuir is a best-fit model with the experimental data.



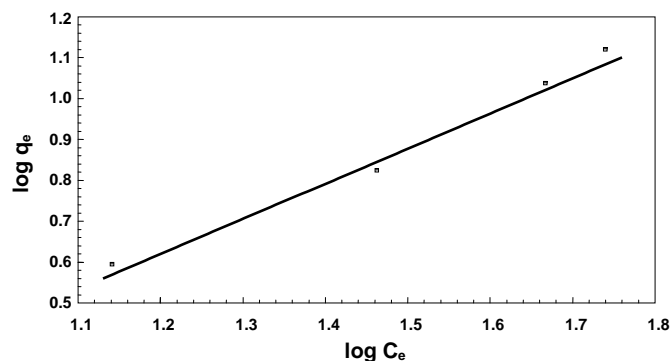
**Fig-8:** Langmuir Isotherm for Reactive blue-4 removal onto MSAC. At adsorbent dose 10 g/l, pH 7, temp. 32°C

### 4.3 FREUNDLICH ISOTHERM

The linear Freundlich isotherm is expressed as:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \tag{9}$$

Figure 9 shows that linear plot of log q<sub>e</sub> vs. log C<sub>e</sub> of reactive blue-4 onto MSAC also follows freundlich isotherm. The freundlich constant value, 1/n (0.19) and correlation coefficients, R<sup>2</sup> (0.974) are reported in Table 2. The value of 1/n has been found to lie between zero and one, indicating dye was favorably adsorbed onto MSAC. However, Freundlich isotherm is less favorable than Langmuir isotherm because R<sup>2</sup> value is low.



**Fig-9:** Freundlich Isotherm for Reactive blue-4 removal onto MSAC. At adsorbent dose 10 g/l, pH 7, temp.32°C

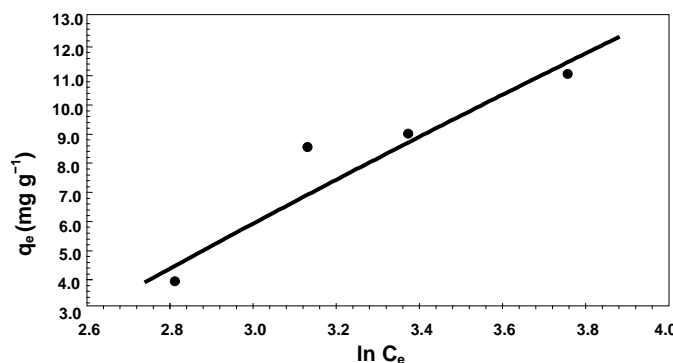
### 4.4 Temkin isotherm

Temkin and Pyzhev studied the heat of adsorption and the adsorbent–adsorbate interaction on surfaces. The Temkin isotherm equation is given as:

$$q_e = B_1 \ln K_T + B_1 \ln C_e \tag{10}$$

Where, B<sub>1</sub> = RT/b, T is the absolute temperature, R is the universal gas constant (8.314 J/mol). In eq. (10) K<sub>T</sub> is the equilibrium binding constant, and B<sub>1</sub> is related to the heat of adsorption.

To check the suitability of Temkin model plots of q<sub>e</sub> vs. ln C<sub>e</sub> has been drawn as shown in Fig.10. Values of various constants along with the correlation coefficients are given in Table 2. Moderately high R<sup>2</sup> values (0.888) confirm that Temkin isotherms provide a reasonable model for the this study.



**Fig-10:** Temkin isotherm for removal of reactive blue-4 onto MSAC. At adsorbent dose 10g/l, temp. 32°C, initial conc. 150 mg/l, pH 7

### 4.5 Dubinin-raduskevich isotherm

Dubinin–Radushkevich isotherm [1] is generally applied to express the adsorption mechanism with a Gaussian energy distribution onto a heterogeneous surface. The linear form of Dubinin and Radushkevich isotherm equation can be expressed as:

$$\ln q_e = \ln Q_S - B \epsilon^2 \tag{11}$$

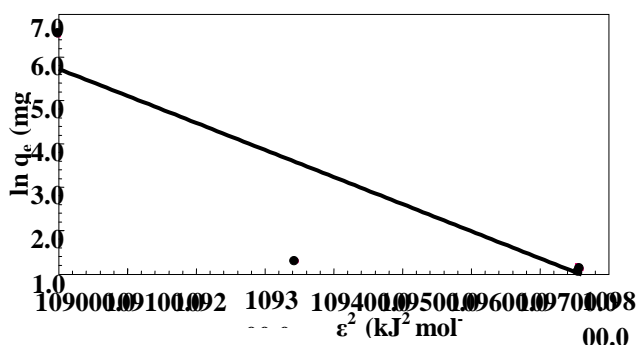
Where,  $\epsilon$  is the Polanyi potential and is equal to:

$$\epsilon = RT \ln \left[ 1 + \frac{1}{C_e} \right] \tag{12}$$

The value of sorption energy, E, can be correlated to  $\beta$  using the following relationship eq. (13):

$$E = \frac{1}{\sqrt{-2\beta}} \tag{13}$$

Polanyi sorption potential,  $\epsilon$  is the work required to remove a molecule to infinity from its location in the sorption space, independent of temperature. Plot for D-R isotherm for reactive blue-4 has been shown in fig 11 and the values of the related constants and correlation coefficients are recorded in Table 2. Poor R<sup>2</sup> value (0.791) indicates that the D-R isotherm cannot be used satisfactorily to fit the present experimental data [7].



**Fig-11:**Dubinin–Radushkevich(D–R) isotherm for removal of Reactive blue-4 onto MSAC. At adsorbent dose 10g/l, temp. 32 °C, initial conc.150 mg/l, pH 7

**Table 2.** Constant values of kinetic models and adsorption isotherms for Reactive blue-4 onto MSAC

Pseudo-first order	$k_i$ 0.005	$R^2$ 0.562	-	-
Pseudo-second order	$k$ 1.45	$h$ 28.2	$R^2$ 0.998	-
Intra-particle diffusion	$k_{id1}$ 4.45	$I$ 88.21	$R^2$ 0.995	-
	$k_{id2}$ 0.014	$I$ 80.2	$R^2$ 0.985	-
Langmuir Isotherm	$Q_m$ 25.8	$K_L$ 0.08	$R_L$ 0.0013	$R^2$ 0.999
Freundlich Isotherm	$K_f$ 3.19	$1/n$ 0.19	$R^2$ 0.974	-
Dubinin Radushkevich(D–R)	$Q_s$ 13.87	$B \times 10^{-6}$ 4.4	$E$ 2.05	$R^2$ 0.791

Temkin Isotherm	$K_T$ 0.30	$B_1$ 6.51	$R^2$ 0.888	-
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### 5. CONCLUSION

The present study shows that the mustard stalk activated carbon (MSAC) immobilized by *Pseudomonas putida* MTCC 1194 is an effective adsorbent for the removal of reactive blue-4 from aqueous solution. The high removal (70.2%) of reactive blue-4 was possible at optimum adsorbent dose 10 g/l, pH 7, concentration 150 mg/l of solution at contact time of 360 min. Mustard stalk activated carbon showed competitive properties as an adsorbent. The kinetics of reactive blue-4 adsorption-biodegradation nicely followed second-order rate expression and demonstrated that intra particle diffusion plays a significant role in the adsorption-biodegradation mechanism. Experimental data for reactive blue-4 on MSAC were best represented by the Langmuir isotherm. This study shows that low cost activated carbon prepared from the mustard stalk along with *P. putida* efficiently decolorized reactive blue-4 from aqueous solution. Hence simultaneous adsorption and biodegradation becomes a viable treatment for removal of dyes.

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