BIOSORPTION OF ACID RED 88 ONTO *AZOLLA PINNATA*: EQUILIBRIUM AND KINETIC STUDIES

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ABSTRACT

Azolla pinnata, a fresh water macro alga, was tested for its ability to remove acid red 88 (AR88) from aqueous solution. Batch equilibrium tests at different pH conditions showed that at pH 3, a maximum AR88 uptake of 55.0 mg/g was observed according to the Toth model. The ability of *A. pinnata* to biosorb AR88 in a packed column was investigated, as well. The experiments were conducted to study the effect of important design parameters such as bed height flow rate and initial dye concentration. At optimum bed height (25 cm), flow rate (5 mL/min) and initial dye concentration (100 mg/L), *A. pinnata* exhibited for AR88. The Thomas model was used to analyze the experimental data and the model parameters were evaluated.

KEYWORDS : *Azolla pinnata*, non linear isotherms, biosorption, packed bed column

I. INTRODUCTION

Biological processes such as biosorption [1] bioaccumulation [2] and biodegradation [3] have been proposed as having potential application in removal of dyes from textile wastewater. Among these, biosorption is more advantageous for water treatment because in this process dead organisms are not affected by toxic wastes, they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles [4]. Biosorption, the passive uptake of pollutants from aqueous solutions by the use of non-living microbial biomass, is a popular technique used for dye removal from wastewaters [5,6]. Some important biosorbents used for dye removal include bacteria [7], fungi [8] and algae [9]. Limited numbers of studies are available on the biological treatment by algal species [5] in spite of their ubiquitous distribution and their central role in the fixation and turnover of carbon and other nutrient elements. However, no research attention has been focused on utilization of macro algae for dye removal. Macro fresh water algae, a renewable natural biomass proliferates ubiquitously and abundantly in the many parts of the world. Azolla, one of the most commonly available fresh water algae, was the focus of the present study. This algae is commonly found in ditches, ponds and slow moving streams and is capable of colonizing rapidly to form dense mats over water surfaces thus imposing negative effects on the aquatic ecology [10].

The present work investigated the dye biosorption behavior of Azolla pinnata on acid red 88 (AR88).

II. MATERIALS AND METHODS

A Azolla pinnata

A. pinnata was collected from Agricultural University, Coimbatore, India. It was then sun dried and crushed to particle sizes in the range of 1 to 2 mm. The crushed particles were then treated with 0.1 M HCl for 5 h followed by washing with distilled water and then kept for shaded dry. The resultant biomass was subsequently used in sorption experiments. Acid red 88 was purchased from Sigma-Aldrich Corporation, Bangalore, India.

B Batch experiments

Batch biosorption experiments were performed in a rotary shaker at 150 rpm using 250 ml Erlenmeyer flasks containing 0.2 g Azolla biomass in 50 ml of solution containing different acid dye concentrations at desired pH conditions (using 0.1 M HCl and 0.1 M NaOH). After 12 h, the reaction mixture was centrifuged at 3000 rpm for 10 min. The dye content in the supernatant was determined using UV-Spectrophotometer (Hitachi, Japan) at respective $_{max}$ values 503 nm for AR88. The amount of dye biosorbed was calculated from the differences between the dye quantity added to the biomass and the dye content of the supernatant using the following equation:

$$q_e = (C_0 - C_e) * V / M \qquad (1)$$

where q_e is the dye uptake (mg/g); C_0 and C_e are the initial and equilibrium dye concentrations in the solution (mg/L), respectively; V is the solution volume (L); and M is the mass of biosorbent (g).

For kinetic experiments, samples were taken at regular time intervals and analyzed for dye concentration. To evaluate the differences in the biosorption rates and uptakes, the kinetic data were described with Pseudo-first and Pseudo-second order models. The linearized form of Pseudo-first and Pseudo-second order model [6] are shown below as Eqs. (2) and (3), respectively.

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t \qquad (2)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \qquad (3)$$

where q_t is the amount of dye sorbed at time t (mg/g) and k_1 is the first order rate constant (1/min); k_2 is the second order rate constant (g/mg min).

C Biosorption isotherm models

Four equilibrium isotherm models were used to fit the experimental data. These isotherms are the following:

Langmuir: $\quad q = \dfrac{q_{max} b C_f}{1 + b C_f} \qquad (4)$

Freundlich : $q = K_F C_f^{1/n} \qquad (5)$

Toth : $\quad q = \dfrac{q_{max} b_T C_f}{[1 + (b_T C_f)^{1/n_T}]^{n_T}} \qquad (6)$

Khan : $q = \dfrac{q_{max} b_K C_f}{(1 + b_K C_f)^{a_K}} \qquad (7)$

where q_{max} is the maximum dye uptake (mg/g), b is the Langmuir equilibrium constant (L/mg), K_F is the Freundlich constant (L/g), n is the Freundlich constant, b_T is the Toth model constant and n_T the Toth model exponent; b_K is the Khan model constant and a_K the Khan model exponent. All the model parameters were evaluated by non-linear regression using MATLAB® software.

D Column experiments

Continuous flow sorption experiments were conducted in a glass column (2 cm internal diameter and 35 cm height). At the top of the column; an adjustable plunger was attached with a 0.5 mm stainless sieve. At the bottom of the column, a 0.5 mm stainless sieve was attached followed by glass wool. A 2 cm high layer of glass beads (1.5 mm in diameter) was placed at the column base in order to provide a uniform inlet flow of the solution into the column.

A known quantity of *A. pinnata* was packed in the column to yield the desired bed height of the sorbent. Dye solution of known concentration was pumped upward through the column at a desired flow rate by a peristaltic pump (Miclins). The concentration of dye at the vent of the column was collected at regular time intervals.

The breakthrough time (t_b, the time at which dye concentration in the effluent reached 1 mg/L) and bed exhaustion time (t_e, the time at which dye concentration in the effluent reached the inlet condition) were used to evaluate the breakthrough curves. The slope of the breakthrough curve (dc/dt) was determined from t_b to t_e. The total quantity of dye mass biosorbed in the column (m_{ad}) is calculated from the area above the breakthrough curve (outlet dye concentration (C) vs. time (t)) multiplied by the flow rate. Dividing the dye mass (m_{ad}) by the sorbent mass (M) leads to the uptake capacity (q) of the alga [11]. Effluent volume (V_{eff}) can be calculated as follows [12]:

$$V_{eff} = F.t_e \times \frac{60}{1000} \qquad (8)$$

where F is the volumetric flow rate (mL/min).

Total amount dye sent to column (m_{total}) can be calculated as follows [12]:

$$m_{total} = \frac{C_0 F t_e}{1000} \qquad (9)$$

where C_0 is the inlet dye concentration (mg/L).

Total dye removal percent with respect to flow volume can be calculated as follows [12]:

$$Total\ dye\ removal\ (\%) = \frac{m_{ad}}{m_{total}} \times 100 \qquad (10)$$

All continuous experiments were conducted at room temperature (30⁰C).

E. Modeling of column data

The column biosorption data obtained at different bed heights, flow rates and dye concentrations were fitted using the Thomas model. The linearized form of Thomas model can be expressed as follows (13) :

$$\ln\left(\frac{C_0}{C} - 1\right) = \frac{k_{Th} Q_0 M}{F} - \frac{k_{Th} C_0}{F} V \qquad (11)$$

where k_{Th} is the Thomas model constant (L/mg h), Q_0 is the maximum solid-phase concentration of solute (mg/g), V is the throughput volume (L). The model constants k_{Th} and Q_0 can be determined from a plot of $\ln[(C_0/C)\text{-}1]$ against t [12].

III. RESULTS AND DISCUSSION

A. Batch Kinetic studies

The experimental results of acid red 88 biosorption as a function of time on *A. pinnata* at various initial concentrations have been shown in Fig. 1. For AR 88, biosorption rate was slow and also the equilibrium time attainment increased with increasing dye concentration. On increasing the initial dye concentrations, the total dye uptake increased and the total percent removal decreased. On changing initial AR88 concentrations from 10 to 1000 mg/L, the amount sorbed increased from 1.17 to 45.6 mg/g at pH 3. But the removal efficiency decreased from 46.8 to 18.2% as the AR88 concentration increases from 10 to 1000 mg/L.

This is because at lower concentration, the ratio of the initial moles of dye molecules to the available surface area is low and subsequently the fractional sorption becomes independent of initial concentration.
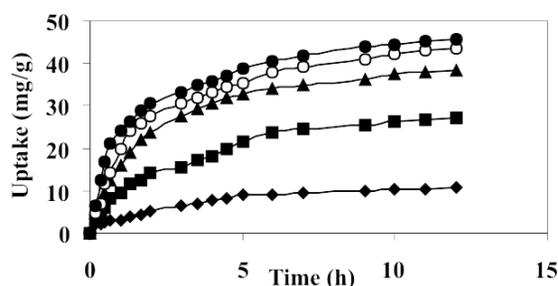

Fig .1 Effect of initial dye concentration on uptake capacity of A. pinnata. Initial dye concentration: (♦) 100 mg/L; ( ) 300 mg/L; ( ) 500 mg/L; ( ) 700 mg/L; ( ) 1000 mg/L.

However, at higher concentration the available sites of sorption becomes fewer compared to the moles of dye present and hence the percentage dye removal is dependent upon the initial dye concentration. It can be concluded that the rate of AR88 binding with alga biomass is more at initial stages, which gradually decreases and remains almost constant after an optimum period of 6-7 h.

The rate constants, predicted equilibrium uptakes and the corresponding correlation coefficients for all concentrations tested have been calculated and summarized in Table 1. For AR88, correlation coefficients were found to be above 0.959, but the calculated q_e is not equal to experimental q_e, suggesting the insufficiency of Pseudo-first-order model to fit the kinetic data for the initial

concentrations examined. The reason for these differences in the q_e values is that there is a time lag, possibly due to a boundary layer or external resistance controlling at the beginning of the sorption process.

The Pseudo-second order model is based on the sorption capacity on the solid phase. Contrary to other well-established models, it predicts the behavior over the whole range of studies and it is in agreement with the binding mechanism being the rate-controlling step.

Table-1. Pseudo-first Order Model Constants

C_0	$(q_e)_{exp}$	Pseudo-first order		
(mg/L)	(mg/g)	k_1	q_e	R^2 †
		(1/min)	(mg/g)	
100	10.6	0.338	10.5	0.971
300	27.2	0.363	48.7	0.964
500	38.3	0.359	33.7	0.980
700	43.6	0.344	38.3	0.959
1000	45.8	0.374	38.3	0.961

This was consistent with the better results obtained with the Pseudo-second order model (Table 2). Correlation coefficients were always greater than 0.992 and the lowest correlation coefficient in this case was better than the first order model correlation coefficients. The values of predicted equilibrium sorption capacities showed good agreement with the experimental equilibrium uptake values.

B. Batch studies

Experimental AR88 biosorption isotherms for *A. pinnata* at different pH conditions (2-5) and temperature (25-35°C) are presented in Fig. 2. Initial solution pH played a relatively significant role in dye biosorption, with maximum AR88 biosorption observed at pH 3. A slightly decreased AR88 biosorption at other pH values may be due to nature of binding groups present in the alga.

Table-2. Pseudo-second Order Model Constants

C_0	$(q_e)_{exp}$	Pseudo-second order		
(mg/L)	(mg/g)	k_2	q_e	R^2
		(g/mg min)	(mg/g)	
100	10.6	0.022	13.7	0.994
300	27.2	0.010	34.1	0.992
500	38.3	0.014	43.5	0.999
700	43.6	0.012	49.0	0.998
1000	45.8	0.017	49.5	0.997

The temperature affected the AR88 biosorption capacity of *A. pinnata*. Among the temperature conditions examined, room temperature (30^0C) favored biosorption. Changing the temperature by $\pm 5^0$C from the room temperature resulted in decreased AR88 biosorption capacity.
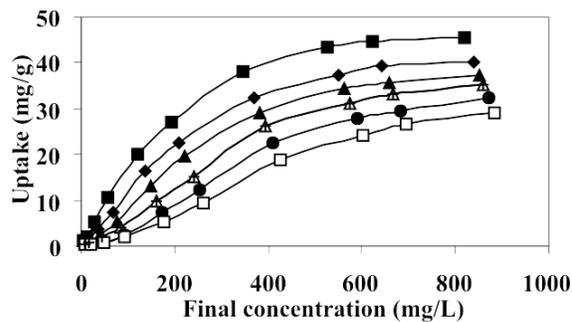

Fig .2 Biosorption isotherms of AR88 for A. pinnata at different conditions (biomass dosage = 4 g/l and agitation rate = 150 rpm). pH and temperature: () 2 and 30^0C; ( ) 3 and 30^0C; ( ) 4 and 30^0C; () 5 and 30^0C; (+) 3 and 25^0C; ( ) 3 and 35^0C; ( ) 3 and 40^0C; respectively

Langmuir, Freundlich, Toth and Khan models were used to describe the non-linear equilibrium between the sorbed AR88 on the alga (q) and AR88 in the solution (C_f). The main reason for extended use of these isotherm models is that they incorporate constants that are easily interpretable. Langmuir sorption model served to estimate the maximum dye uptake values where they could not be reached in the experiments. Table 3. - shows maximum AR88 uptake of 53.76 mg/g was obtained at optimum pH (3) and temperature (30^0C)

Table-3. Langmuir Model Parameters At Different pH Conditions

Temp	pH	Langmuir model		
(^{0}C)		Q_{max} (mg/g)	b (L/mg)	R^2
30	2.0	50.76	0.003	0.998
30	3.0	53.76	0.004	0.995
30	4.0	45.87	0.002	0.993
30	5.0	42.02	0.001	0.990
25	3.0	42.02	0.001	0.990
35	3.0	37.04	0.001	0.996
40	3.0	36.36	0.001	0.993

To a lesser extent, the Freundlich model also described the equilibrium data. Both Freundlich constants (*K* and *1/n*) also reached their maximum values at pH = 3.0 and temperature = 30^0C (Table 4).

Table-4. Freundlich Model Parameters At Different pH Conditions

Temp	pH	Freundlich model		
(^{0}C)		K_F (L/g)	n	R^2
30	2.0	0.330	1.20	0.989
30	3.0	0.483	1.26	0.970
30	4.0	0.304	1.20	0.985
30	5.0	0.220	1.15	0.982
25	3.0	0.220	1.14	0.963
35	3.0	0.174	1.11	0.972
40	3.0	0.155	1.10	0.974

In the case of Toth model, the maximum uptake was obtained in pH 3 and 30^0C. The other two constants b_T and n_T were also maximum at pH 3 and at temperature 30C. The correlation coefficients in the range of 0.995 to 0.999 were obtained and the model parameters varied with the

conditions examined. The model reasonably fits the equilibrium data at all conditions examined. According to the Khan model constant, q_{max}, observed were maximum at the optimum condition (pH 3.0 and 30^{0}C), whereas other two constants (b_K and a_K) lowest at this condition (Table 6). A typical example of batch sorption isotherm fitted using four examined models is shown in Fig .3.

D. Column studies

Biosorption of AR88 by *A. pinnata* was presented in the form of breakthrough curves.
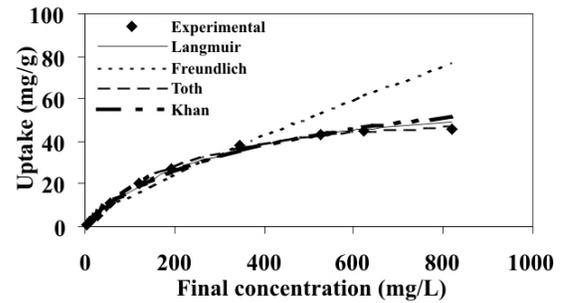

Fig .3. Application of isotherm models to experimental isotherm data obtained during AR88 biosorption by A. pinnata (pH = 3, tempera ture = 30^{0}C, agitation rate = 150 rpm, biosorbent dosage = 4 g/l).

Table-5. Toth model parameters at different pH conditions

Temp (^{0}C)	pH	Toth model			
		q_{max} (mg/g)	b_T	n_T	R^2
30	2.0	52.5	0.002	0.697	0.995
30	3.0	55.0	0.004	0.770	0.999
30	4.0	49.2	0.003	0.698	0.995
30	5.0	47.6	0.002	0.564	0.996
25	3.0	48.5	0.003	0.745	0.997
35	3.0	46.2	0.002	0.689	0.998
40	3.0	45.9	0.002	0.623	0.995

Fig .4 shows the breakthrough profile of AR88 biosorption for different bed heights (15, 20 and 25 cm). In order to yield different bed heights, 4.52, 6.14 and 7.89 g of biomass were added to produce 15, 20 and 25 cm, respectively

Table-6. Khan model parameters at different pH conditions

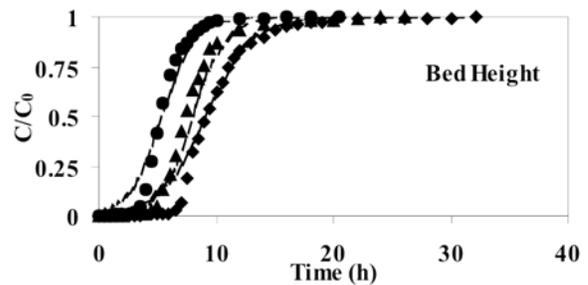| Temp (^{0}C)	pH	Khan model			
		q_{max} (mg/g)	b_K	a_K	R^2
30	2.0	45.2	0.003	0.789	0.962
30	3.0	48.0	0.005	0.802	0.987
30	4.0	46.5	0.003	0.854	0.965
30	5.0	44.2	0.002	0.792	0.956
25	3.0	46.2	0.004	0.812	0.987
35	3.0	44.7	0.003	0.645	0.968
40	3.0	42.3	0.002	0.689	0.979

Fig .4. Breakthrough curves for AR88 biosorption onto A. pinnata biomass at different bed heights (flow rate = 5 mL/min, initial AR88 concentration = 100 mg/L, pH = 3). Bed heights: ( ) 15 cm; ( ) 20 cm; (♦) 25 cm. (------) Predicted from Thomas model.

Fig .5 shows the influence of flow rate on biosorption of AR88 by *A. pinnata* by keeping initial dye concentration (100 mg/L) and bed height (25 cm) constant and varying the flow rate from 5 to 15 mL/min. In contrast to bed height results, the column performed well at lowest flow rate. Earlier breakthrough time appeared for highest flow rate, resulting in low uptake and least % removal. This behavior may be due to insufficient time for the solute inside the column and the diffusion limitations of the solute into the pores of the sorbent at higher flow rates [14].
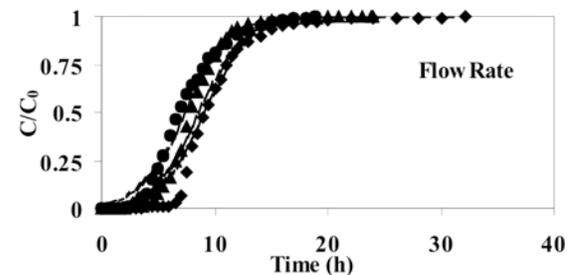

Fig .5. Breakthrough curves for AR88 biosorption onto A. pinnata biomass at different flow rates (bed height = 25 cm, initial AR88 concentration = 100 mg/L, pH = 3). Flow rates: (♦) 5 mL/min; ( ) 10 mL/min; ( ) 15 mL/min. (------) Predicted from Thomas model.

The breakthrough curves obtained by changing AR88 concentration from 50 to 100 mg/L at 5 mL/min flow rate and 25 cm bed height are shown in Fig .6. At the highest AR88 concentration (100 mg/L) the *Azolla* bed saturated quickly leading to earlier breakthrough and exhaustion

time. Fig .6, shows that highest uptake and high percentage dye removal are obtained at the highest dye concentration. Also more positive and steep breakthrough curve was obtained for 100 mg dye/L. The driving force for biosorption is the concentration difference between the dye on the biosorbent and the dye in the solution [22]. Thus the high driving force due to the high AR88 concentration resulted in better column performance. Comparison of experimentally determined and Thomas model predicted breakthrough curves are shown in Figs. 4, 5 and 6. As bed height increased, the values of Q_0 increased and the values of k_{Th} decreased. The bed capacity Q_0 decreased and Thomas constant k_{Th} increased with increasing flow rate. In general, good fits were obtained in all cases with correlation coefficients ranging from 0.992 to 0.999 for AR88.
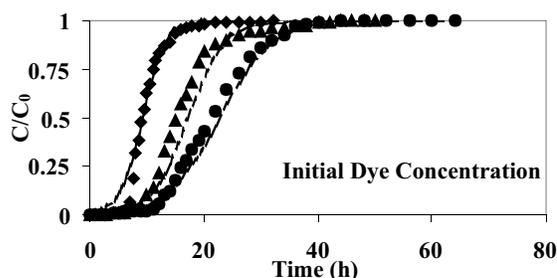

Fig .6 Breakthrough curves for AR88 biosorption onto A. pinnata biomass at different dye concentrations (bed height = 25cm, flow rate = 5mL/min, pH = 3). Initial AR88 concentrations: ( ) 50 mg/L; ( ) 75 mg/L; (♦) 100 mg/L. (------) Predicted from Thomas model.

IV. SUMMARY AND CONCLUSION

The present study investigated the following features of dye biosorption on deactivated macro blue green fresh water alga *A. pinnata* in a batch reactor and in a packed bed column. Batch experiments provided fundamental information regarding optimum pH and maximum dye uptake. Langmuir and Toth model isotherms fit well with experimental data. Column experiments were performed in a packed column, as it makes the best use of the concentration difference known to be a driving force for adsorption. Thus, *A. pinnata* possesses all intrinsic characteristics to be employed for the treatment of Acid red 88 bearing industrial effluents.

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