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Investigation on Biosorption of Reactive Blue 140 by Dead Biomass of *Aspergillus niger* HM11: Kinetics and Isotherm Studies

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Abstract: The biosorption equilibria and kinetics of Reactive blue 140 were examined in this study using dead fungal biomass of Aspergillus niger HM11. The results gained from this study were described by Langmuir isotherm model better than Freundlich isotherm models to the biosorption equilibrium data. The second-order kinetic model by Ho and Mckay described well the experimental data. Studies on pH effect and desorption show that chemisorption seems to play a major role in the adsorption process. The maximum adsorption capacity was calculated for dead biomass indicating that dead biomass can be considered as a good sorbent material for Reactive blue 140 solution since autoclaved biomass is much safer as it does not pose any threat to environment.

Key words: Biosorption · Reactive blue 140 · *Aspergillus niger* HM11 · Isotherms · Kinetics · SEM

INTRODUCTION

Dyes usually have a synthetic origin and complex aromatic molecular structures which possibly come from coal-tar based hydrocarbons such as benzene, naphthalene, anthracene, toluene and xylene [1]. There are more than 100,000 commercially available dyes with over $7x\ 10^7$ tons of dyestuff produced annually worldwide [2-3]. According to the statistics, India, the former USSR, Eastern Europe, China, South Korea and Taiwan consume approximately thousand tons (kt) of dyes annually [4]. Textile dyeing industries in Tirupur and Karur of Tamil Nadu (India) discharge effluents ranging between 80 and $200m^3/t$ of production [5].

The dyes present in textile effluent impart persistent color to the receiving streams and interfere with photosynthesis of the phytoplankton [6] and this leads to chains of adverse effects on the aquatic eco-system, as the growth of primary as well as secondary and tertiary consumer is adversely affected. Additionally, toxic degradation products can be formed. These chemicals are not only poisonous to humans but also found toxic to aquatic life [7] and they may result in food contamination [8]. A very hopeful area for removing unwanted color from textile wastewater is biotreatment that is embattled at breaking down the dye molecules to basic elements (mineralizing). Interest is therefore now focused on the microbial biodegradation of dyes as a better alternative [9].

Fungal systems appear to be most appropriate biological agent in the treatment of colored and metallic effluents [10]. In recent years, several adsorbents have been identified as possessing good dye-binding capabilities [11-14]. In particular, biomaterials of microbial origin have been very effective because of their cell wall constituents. Important fungal biosorbents include *Aspergillus* [15], *Penicillium* [16] and *Rhizopus* [17-18]. Keeping this in mind, the present study has been designed to utilize *Aspergillus niger* HM11, a promising fungal agent for biosorption of Reactive blue 140 from aqueous solution.

MATERIALS AND METHODS

Dye stuff: Dye was procured from a textile industry situated in Coimbatore, Tamil Nadu, India. The dye used was of commercial grade and used without further purification.

Isolation and taxonomic identification of the fungus: The fungal strain was isolated from the soil of dye contaminated industrial site located in Coimbatore district,

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Tamilnadu, India. Further confirmation of taxonomic identification of the fungal isolate was carried out at Fungus Identification Service, Mycology and Plant Pathology Laboratory, Agharkhar Research Institute, Pune, India.

Biosorption studies

Preparation of fungal biomass: Five days old *Aspergillus niger* HM11 spores of 0.6 cm (Ref 3.9.1) suspension was taken and inoculated into 50 ml of sterile minimal media (pH 6.0) in 100 ml Erlenmeyer flaks and incubated at 30°C for 24 hrs under shaking at 150rpm. After good growth, the mycelium developed as pellets was separated by filtration through Whatmann.No.1 filter paper and washed with generous amount of de-ionized water until free from the media components. This was used for the preparation of dead biomass.

Dead biomass: The washed mycelial pellets were subjected to autoclaving for 30 min at 121°C and used as dead biomass.

Preparation of the Adsorbate: The stock solution of 1000 mg/L of Reactive blue 140 was prepared. From the stock solution, various concentrations of working dye solutions (10, 20, 30, 40, 50 and 60 mg/L) were prepared.

Adsorption Experiments

Batch mode studies: Batch mode experiments were carried out to investigate factors such as agitation time, initial concentration of dyes, dosage of the biosorbent and pH influencing the rate and extent of uptake of Reactive blue 140 by dead fungal biosorbent.

Effect of pH: The adsorbate solutions (50 ml in 100 ml conical flasks) were prepared at various levels of pH (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0) using either 1.0 N HCl or 1.0 N NaOH. The dye solutions were agitated with optimum dosages of adsorbents (0.5 g of dead biomass). The amount of dye adsorbed was calculated. The optimum pH was determined from the plot drawn with pH against percent dye removal.

Effect of Agitation time and Initial dye concentrations: Fifty ml of Reactive blue 140 at various concentrations (10, 20, 30, 40, 50 and 60 mg/L) were taken in 100 ml conical flasks. To the aqueous solutions, one gm of adsorbent (dead biomass) was added and the flasks were agitated on a rotary shaker (150 rpm) at 30°C. The flasks were withdrawn at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 mins. The adsorbent was separated by centrifugation at 10000 rpm for 15 mins. The concentration of remaining adsorbate was determined. From this, the amount adsorbed was calculated. A plot was drawn to determine the optimum contact time to obtain equilibrium in adsorption. Control experiments were carried out without adsorbent to estimate the adsorbate removal due to adsorption on to the walls of the flasks.

Effect of Adsorbent dosage: The adsorbate solutions (50 ml) were agitated with various dosages of dead biomass of *A.niger* HM11 (0.1, 0.2, 0.3, 0.5, 0.75 and 1.0g / 50 ml) at 150 rpm at 30°C for the equilibrium period. After the equilibrium period, the adsorbents and the adsorbate were separated and the amount adsorbed was determined. A graph was plotted with adsorbent dosage vs percent adsorbate removal. The optimum adsorbent dosage for adsorbent removal was determined. The data obtained in the adsorption studies were used for the determination of Langmuir and Freundlich isotherms and for Lagergren rate constant and the second order rate kinetics.

Measurement of decolorization: The absorption spectra of the clear supernatant were recorded at $\lambda_{max.}$ (612nm) using a spectrophotometer (UV-Vis 3210, Hitachi, Japan). Medium containing dyes without the inoculum was taken as control and the experiments were carried out in triplicates. The initial and final absorbance values obtained were then used to calculate percentage decolorization of the dye:

$$\frac{\text{Initial absorbance value-Final absorbance value}}{\text{Initial absorbance value}}$$

Desorption studies: This was carried out with adsorbate loaded adsorbents obtained from batch processes, in which the adsorbate solutions (50 mg/L) were treated with optimum dosage of adsorbents for optimum contact time. The pellets were washed gently with distilled water to remove unabsorbed dyes. After washing, one set of dye adsorbed dead samples was resuspended in flask containing 50 ml of distilled water and monitored at different time intervals of 0, 15, 30, 60, 90, 120 and 150 mins using 1.0 N NaOH as a desorption agent and agitated for the equilibrium time of respective adsorbate.

Adsorption isotherms and kinetics: The Langmuir plot was obtained using the equilibrium time curves data

(i.e. the adsorbate concentration was varied, while the adsorbent dose was fixed). Freundlich plots were obtained from the equilibrium data of the adsorbent dose effect (i.e. the adsorbate concentration was fixed, while the adsorbent dose was varied). Kinetic studies were also carried out with different initial concentrations of Reactive blue 140, while maintaining the adsorbent dosage at a constant level.

SEM Analysis: Scanning electron microscopy of dead biomass before and after biosorption was carried out on a JEOL/EO, JSM-5610 (Japan) SEM. After biosorption, the biomass was collected and washed, dried overnight at 60° C. The biomasses were then glued separately on a brass stub using 'Spot-O-gold' labels and were coated with gold- palladium using a JEOL, JFC- 1200 (Japan) fine coater under reduced pressure. The images of dead biomass were then captured at 3000 x and 5000 x magnifications using an electron beam high voltage of 20Kv at a 45° C tilt on left side.

RESULTS AND DISCUSSION

Identification of the fungal isolate: The fungal strain was identified by employing Lactophenol cotton blue staining method and based on the morphological features, the strain was identified to be *Aspergillus* sp. Further taxonomic identification of the fungal isolate was carried out at Fungus Identification Service, Mycology and Plant Pathology Laboratory, Agharkhar Research Institute, Pune, India. The fungal isolate was identified as *Aspergillus niger* HM11 and was selected for further biodegradation and biosorption studies.

Effect of pH: pH value of 6.0 was found to be ideal for dead biomass and the maximum percent removal of Reactive blue 140 was 74.29% at a maximum test concentration of 60 mg/L (Figure 1). Dye adsorption on to dead fungal biomass at extreme acidic and alkaline condition, decreased the decolorization of Reactive blue 140 (10-60 mg/L).

Effect of adsorbent dosage: When dead biomass was concerned, an optimum dye removal (74.05-90.02%) was observed at a dosage of 0.3 g/ 50 mL (Figure 2). Minimum and maximum dye removals of 16.98-33.43% and 76.7-92.0% were observed at dosages of 0.1 and 0.5g/ 50 mL, respectively. Consequently the optimum biomass of 0.3g (dead biomass) per 50mL was used for biosorption of Reactive blue 140.

Effect of agitation time and initial dye concentration: The adsorption data of dye concentration versus agitation time on Reactive blue 140 removal by dead biomass (Figure 3) indicated that the uptake of dye increased with increase in agitation time, but remained constant after the equilibrium time period whereas the percent dye removal decreased with an increase in the initial dye concentration in the solution in both types of biomass used.

The equilibrium time required for the maximum removal of Reactive blue 140 by dead fungal biomass was 135 min at all the dye concentrations studied. The percent dye removal at equilibrium time was found to be 93.45% and 77.01% for the dye concentrations of 10 and 60 mg/L respectively.

Desorption profile: The equilibrium time required for the desorption of various Reactive blue 140 concentrations from dead biomass was found to be 120 min. Maximum desorption of 81.11% at 10 mg/L dye concentration and 35.82% at 60 mg/L was observed at 120 mins and a similar pattern of desorption was observed after 120 mins (Figure 4).

Adsorption isotherms

Langmuir isotherm: The Langmuir isotherm is based upon an assumption of monolayer adsorption onto a surface containing finite number of adsorption sites of uniform energies of adsorption with no transmigration of adsorbate on the plane of the surface. Langmuir isotherm is given by [19],

$$q_e = \frac{Qbc_e}{1 + bC_e}$$

However, the linear form of Langmuir equation can be written as:

$$\frac{C_e}{q_e} = \frac{1}{Qb} + \frac{C_e}{Q}$$

Where, C_e is the equilibrium concentration of a dye in solution (mg/L), q_e is the amount of dye sorbed on to fungal biomass (mg/g), Q is the Langmuir constant related to sorption capacity (mg/g), b is the Langmuir constant related to sorption energy (L/mg). Data obtained for the sorption of dye in the concentration range of 10 to 60 mg/L of Reactive blue 140 were fitted to the Langmuir isotherm (Figure 5). A plot of C_e/ q_e versus C_e gave a straight line, the slope and intercept of which correspond to Q and b respectively. The computed

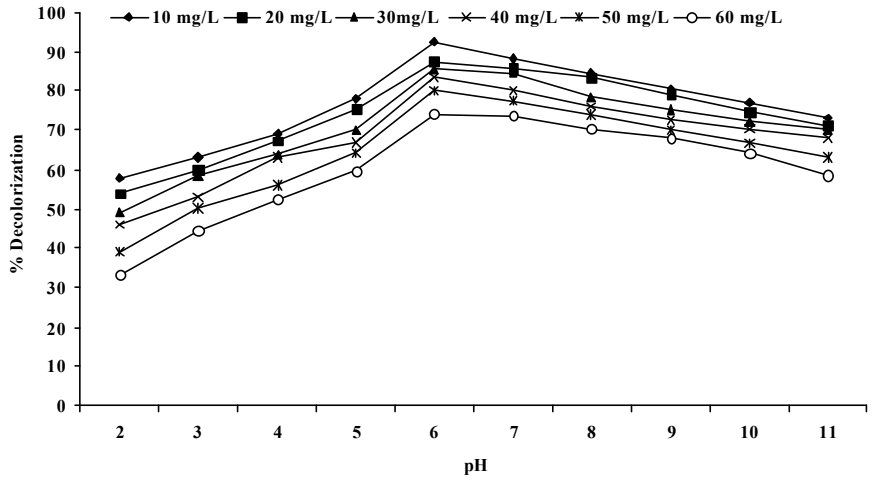

Fig. 1: Effect of pH on the removal of Reactive blue 140 using dead biomass

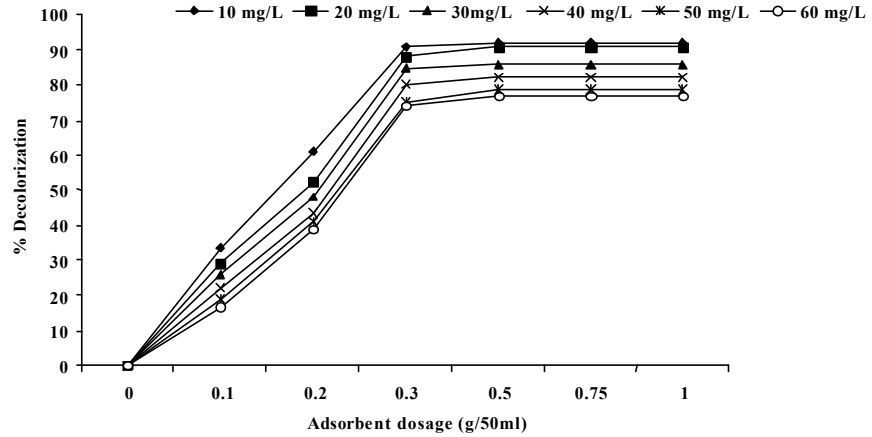

Fig. 2: Effect of adsorbent dosage on the removal of Reactive blue 140 using dead biomass (contact time: 120 min; pH: 6.0; temperature: 300C)

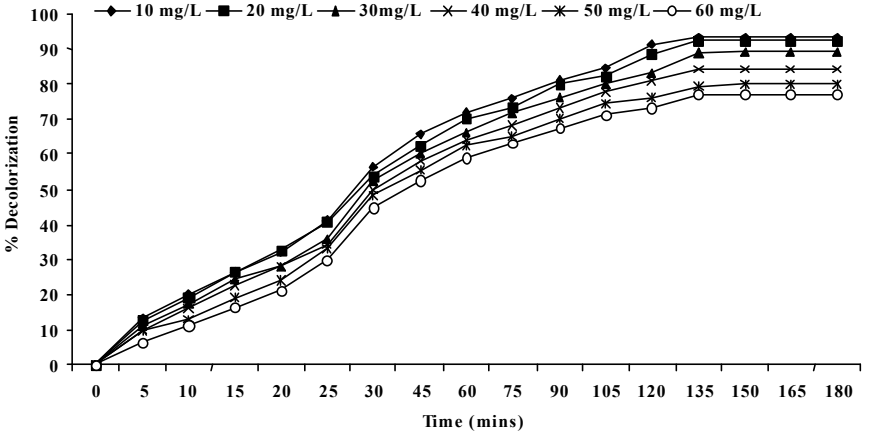

Fig. 3: Effect of agitation time and initial dye concentration on the removal of Reactive blue 140 using dead biomass (adsorbent dosage: 0.3g/50 mL; pH: 6.0; temperature: 300C)

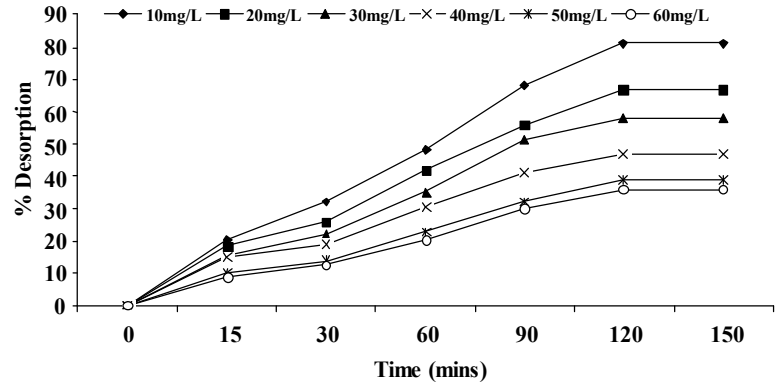

Fig. 4: Desorption profile of Reactive blue 140 from dead biomass

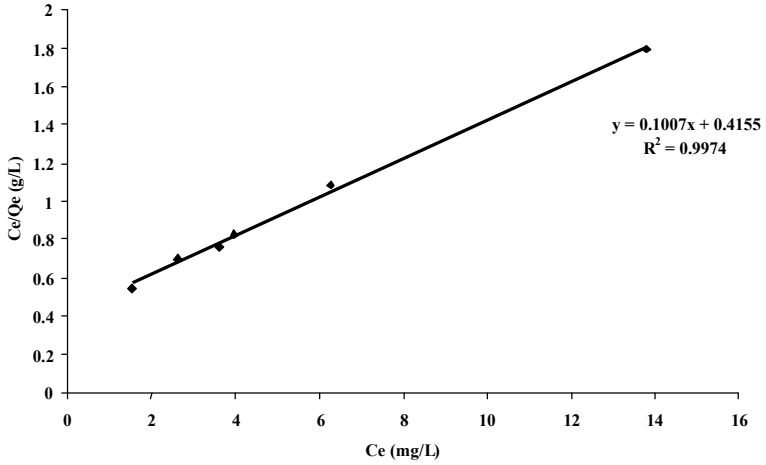

Fig. 5: Langmuir plot for Reactive blue 140 biosorption using dead biomass

Table 1: Langmuir and Freundlich isotherm constants

Langmuir constants			Freundlich constants		
Q(mg/g)	b (L/mg)	R^2	K_f	1/n	R^2
9.9304	0.24235	0.9974	2.4951	0.4382	0.9902

Table 2: Comparison of the RL, R2 and rate constants of Lagergren I order and Pseudo II order kinetic models

			Pseudo I order kinetic model			Pseudo II order kinetic model			
Initial conc. (mg/L)	R_L	q_e(exp) (mg/g)	K_{ad}(1/min)	q_e(cal)(mg/g)	R^2	K_2(g/mg/min)	q_e(cal)(mg/g)	h	R^2
10	0.29210	1.5575	0.016351	8.341	0.9652	0.01070	2.049	0.04492	0.9975
20	0.17103	3.074	0.002994	6.124	0.8815	0.00547	3.979	0.08660	0.9936
30	0.12091	4.339	0.003915	6.896	0.8713	0.00338	4.003	0.05416	0.9342
40	0.09351	5.62133	0.010824	7.997	0.942	0.00774	4.7709	0.17617	0.9786
50	0.07623	6.6758	0.017503	6.209	0.9747	0.00169	9.4607	0.15126	0.9805
60	0.06435	7.701	0.016351	7.338	0.966	0.00093	12.300	0.14069	0.9622

correlation coefficients and the Langmuir constants for Reactive blue 140 are presented in Table 1. The R^2 values were above 0.9974. Examination of the Table showed that the maximum sorption capacity of dead biomass for Reactive blue 140 was 9.9304 mg/g..

Another dimensionless equilibrium parameter (R_L) can be estimated using the following relation. The equilibrium parameter, RL, is used to predict if an adsorption system is ‘‘favorable” or ‘‘unfavorable”. RL > 1.0 (Unfavourable), R L= 1.0 (Linear), 0< RL< 1.0 (Favorable) and RL = 0 (Irreversible).

$$R_L = \frac{1}{1 + bC_o}$$

Where b is the Langmuir constant and C_o is the initial dye concentration in the solution (mg/L). According to Hall *et al.* [20] mathematical calculations show that this parameter gives an indication of type of isotherm. The values of R_L (Table 2) were between 0 and 1 for selected dyes which indicate the applicability of the Langmuir isotherm.

Freundlich isotherm: It is another form of Langmuir approach for adsorption on amorphous surface. It assumes the heterogeneity of surface and the exponential distribution of active sites and their energies [21]. The Freundlich isotherm was tested in the linear form

$$\log\ q_e = \log K_f + 1/n\ \log C_e$$

Where, C_e is the equilibrium concentration of a dye in solution (mg/L), q_e is the amount of dye sorbed on to biomass of *A. niger* HM11 (mg/g) and K_f and 1/n are Freundlich constants.

When log q_e was plotted against log C_e, a linear plot was obtained for each of the dye tested. Freundlich plot obtained for Reactive blue 140 is shown in Figure 6. The Freundlich constants 1/n (Intensity of adsorption) and K_f (Adsorption capacity) were computed from the slope and intercept of the plot. The computed correlation coefficients and Freundlich constants for the dyes studied are presented in the Table 1. The R^2 values were higher than 0.9974, however, the R^2 values for Langmuir equation for Reactive blue 140 were higher than those obtained for Freundlich equation (R^2= 0.9902). The constant 1/n showed the sorption intensity and its fractional value (0<1/n<1) showed the heterogenous nature of sorbent surface. The calculated sorption capacity for dead biomass for Reactive blue 140 was 2.4951.

Adsorption kinetics: The study of adsorption kinetics describes the solute uptake rate and evidently this rate controls the residence time of the adsorbate at the solid solution interface. The rate at which sorption takes place is of most importance when designing batch sorption system. The rate of sorption can be computed from the kinetic study.

In this study the kinetics of Reactive blue 140 biosorption on the adsorbent, *A.niger* HM11 biomass were analysed using pseudo first order [22], pseudo second order [23-24]. The confirmation between experimental data and the predicted values using different models were expressed by the correlation coefficients (R^2 value close or equal to 1).

Lagergrens first order model: For a batch contact time process where the rate of sorption of dyes on to the adsorbent surface is proportional to the amount of dye sorbed from the solution phase, the pseudo first order kinetic equation may be expressed as

$$dq_t / qt = K_{ad}\left(q_e - q_t\right)$$

Where, q and q_e are the amount of dye adsorbed (mg/g) at time t and at equilibrium time, respectively and K_{ad} is the rate constant of adsorption (1/min)

After integration and applying boundary conditions, viz. that the initial conditions are $(q_e-q_t) = 0$ at t = 0, equation (1) becomes:

$$\log\left(q_e - q_t\right) = \log\ q_e - \frac{K_{ad}t}{2.303}$$

Figure 7 showed the plot of linearised form of pseudo first order kinetic model at all concentrations of dyes studied. The slopes and intercepts of plots of log (q_e-q_t) versus t were used to determine the values of pseudo first order rate constant K_{ad} and equilibrium adsorption capacity q_e. The R^2 values for the pseudo first order kinetic plots were lower than those for pseudo second order kinetic plots (Table 2). The calculated rate constants, experimental and predicted q_e with corresponding correlation coefficient values are presented in Table 2. The correlation coefficients for the pseudo first order kinetics model obtained for all concentrations of dyes were low and the predicted q_e values deviated reasonably from the experimental values. The higher R^2 values confirm that the sorption process of dyes onto fungal biomass followed a pseudo first order kinetic model did not fitted well. The rate constants (calculated qe) of pseudo first order increased with an increase in the initial dyes concentration.

Ho's pseudo second order model: The pseudo second order kinetic model was expressed as [23-24]

$$dq_t / qt = K_2\left(q_e - q_t\right)^2$$

Where, K_2 is the pseudo second order rate constant (g/mg/min), q_e and q_t represent the amount of dyes adsorbed (mg/g) at equilibrium and at time t.

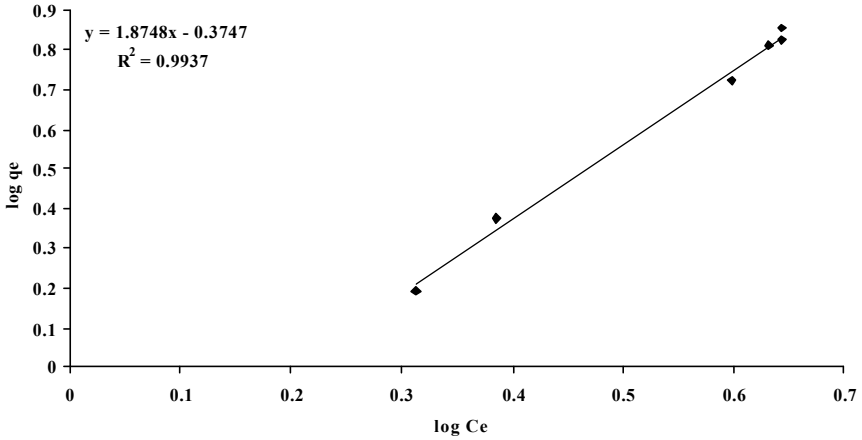

Fig. 6: Freundlich plot for Reactive blue 140 biosorption using dead biomass

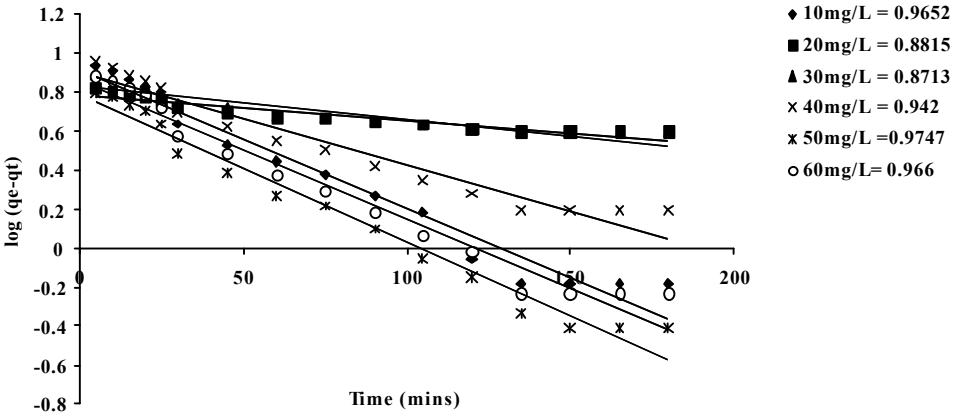

Fig. 7: Pseudo-first order plot for Reactive blue 140 biosorption using dead biomass

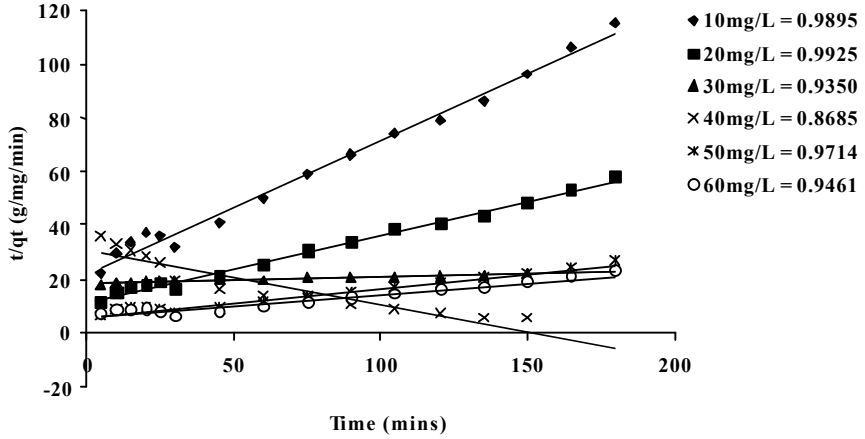

Fig. 8: Pseudo-second-order plot for Reactive blue 140 biosorption using dead biomass

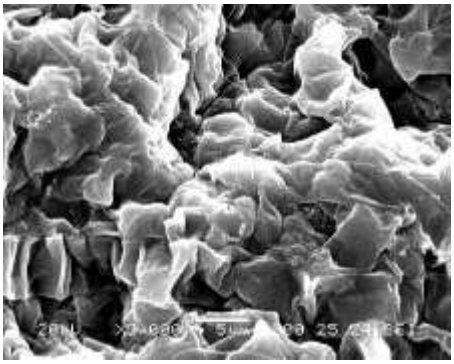

Fig. 9: SEM image of dead biomass of *Aspergillus niger* HM11 at 5000x

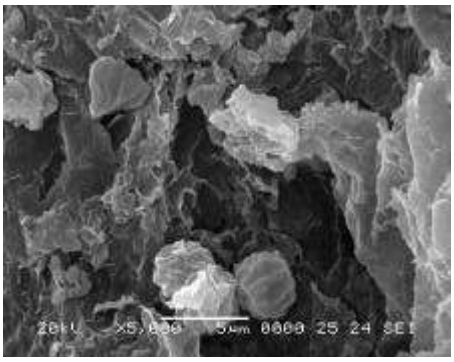

Fig. 10: SEM image of dead biomass of *Aspergillus niger* HM11 after biosorption of Reactive blue 140 at 5000x

For the boundary condition t = 0 to t = t and $q_t = 0$ to $q_t = q_t$, the integral form of the equation (3) becomes

$$q_t = \frac{t}{\frac{1}{K_2 q_e^2} + \frac{t}{q_e}}$$

$$t/q_t = \frac{1}{K_2 q_e^2} + \frac{t}{q_e}$$

The kinetic data were analysed using the equation. The values of the K_2 and q_e presented were calculated from the intercepts and slopes of the plots of t/q_t vs t and the corresponding R^2 values are given in Table 2.

The constant K_2 was used to calculate the initial sorption rate h, at $t \rightarrow 0$ as follows

$$h = K_2 q_e^2$$

The initial sorption rate increased with an increase in the initial dyes concentration for both the dyes which showed no specific trend. The Figure 8 showed the plots of linearised form of pseudo second order kinetic model at all concentrations of dyes studied. The slopes and intercepts of plots of t/qt versus t were used to determine the values of pseudo second order rate constant K_2 and equilibrium adsorption capacity q_e. The correlation coefficients for the pseudo second order kinetics model obtained for all concentrations of dyes were high and the predicted q_e values deviated reasonably from the experimental values. Further, the pseudo first order model did not fit well for the whole range of contact time suggesting that the adsorption system for the tested dyes obeyed the pseudo second order kinetic model.

Scanning electron micrograph studies: The surface morphology of the dead fungal mycelia of *A.niger* HM11 before and after biosorption of Reactive blue 140 was exemplified by the scanning electron micrograph (Figure 9 and 10). Dead biomass was likely to be caked by autoclave treatment, implying that physical strength of hyphae of the fungus was weak and had porous surface. Further Scanning electron micrograph of biomass revealed that there was no noticeable change in the surface morphology of dead biomass of *A.niger* HM11 after biosorption of Reactive blue 140.

DISCUSSION

Despite the existence of a variety of chemical and physical treatment processes, the environment friendly approach in treating textile dyeing effluents has stimulated interest in exploring novel means. The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation [25]. Dye removal by the fungus occurs either through bioaccumulation or biodegradation. The colour of the fungal biomass after dye removal is an indicator of the principal mechanism behind dye removal [26]. Textile dyes vary greatly in their chemistries and therefore their interactions with *microorganisms* depend on the chemistry of a particular dye and the specific chemistry of the microbial biomass [27]. The microbial surface carries various types of functional groups of amino, carboxylate, phosphate and hydroxyl which are responsible for the sequestration of hazardous materials from industrial effluents [28].

In this study, when biosorption of Reactive blue 140 was concerned, even though pH 6.0 was found to be ideal, biosorption capacity of dead biomass of

Aspergillus niger HM11 worked in a broad range of pH (4-11). The report of Maurya *et al.* [29] coincides with our study in which they observed that sorption of Methylene Blue (initial concentration 100 mg/l) by *Fomes fomentarius* and *Phellinus igniarius* increased from 18% to 75% and 16% to 79% respectively when the pH was increased from 3 to11. They stated that this was due to the increase in net electronegativity of the biosorbent due to deprotonation of different functional groups.

The results of the present investigation indicated that an increase in adsorbent dosage did not influence percent removal. Such a pattern was observed by Yesilada *et al.* [30] where they found that increase in the amount of pellet was accompanied by increase in percent decolorization. The adsorption data of the present study indicated the uptake of dye increased with increase in agitation time, but remained constant after the equilibrium time period. Whereas the percent dye removal decreased with an increase in the initial dye concentration for both types of biomass. Sadhasivam *et al.* [31] reported that percent dye removal at equilibrium time decreased from 86.26% to 75.73% for the dye concentrations of 10-50 mg/L. The effect for agitation time on dye uptake is a single, smooth and continuous curve leading to saturation, suggesting the possible monolayer coverage of dye on the surface of the adsorbent [32].

In the present study, 120 mins was found to be the equilibrium time required for desorption of Reactive blue 140 from dead biomass when NaOH (1.0N) was used as an eluent. Bhole *et al.* [33] used HCl and ethanol for the maximum desorption of methyl violet, basic fuchsin and their mixtures from *Aspergillus niger* which was attained at 60 mins.

In this study, maximum adsorption capacity Q_o was 9.9304 mg/g. The same trend was observed in the adsorption of Rhodamine B onto banana and orange peel [34]. Gallagher *et al.* [35] used the Freundlich and Langmuir isotherm models and found that both models fitted the biosorption of reactive dye on *Rhizopus oryzae* which suggested that biosorption involved a hybrid mechanism on a heterogenous surface. A similar phenomena was observed in the adsorption of Acid blue 29 from an aqueous solution by fungus *Aspergillus niger* [36].

The qe values obtained from the first order kinetic equation did not agree with the experimental qe values, which indicated that the adsorption of both the dyes by fungal biomass did not follow the first order rate kinetics. Such a kinetic equation was reported by Sadhasivam *et al.* [31] when *Trichoderma harzianum* mycelial waste was exploited for the removal of Rhodamine 6G from aqueous solution. Similar phenomena have also been observed in biosorption of RB 2, RY 2 and Remazol Black B dyes on biomass [37].

The efficacy of dead biomass was found to be higher due to upper adsorption strength, change in surface property and increase in surface area due to cell rupture after death which was found in autoclaved biomass [36]. Heat treatment can modify surface binding sites via denaturation of proteins on the cell wall structures. In addition, these pores reduce the mass transfer resistance and facilitate the diffusion of dye molecules because of high internal surface area with low diffusional resistance and facilitate mass transfer because of their high internal surface area which implies high adsorption capacity and rate [38].

It was concluded from the study that dead A.niger HM11 in shaking conditions was very effective at removing Reactive blue 140 dye from the aqueous solution since the results obtained from this study are well described by Langmuir isotherm and Freundlich isotherm models.

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