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BIOTECHNOLOGICAL APPROACH TO ASSESS THE PERFORMANCE OF DRIED BIOMASS OF *CANDIDA TROPICALIS* FOR REMOVAL OF BASIC VIOLET 3 FROM AQUEOUS SOLUTIONS

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ABSTRACT
In this study dried biomass of *Candida tropicalis*, is used to remove Basic Violet 3 in a batch process. The influences of operational parameters such as particle size, pH, biosorbent dosage, initial dye concentration on biosorption were investigated. The experimental data were analyzed by the Langmuir, Freundlich and Temkin models of adsorption. The adsorption isotherm data were fitted well to the Langmuir isotherm with monolayer adsorption capacity of 19.53 mg/g. The kinetic data obtained at different initial concentrations were analyzed using pseudo-first–order, pseudo-second-order and intraparticle diffusion equations. The adsorption process was found to follow the pseudo-second-order kinetic model suggesting the adsorption process was controlled by chemisorption. The intraparticle diffusion plots being non linear over whole time range indicated that intra-particle diffusion cannot be the dominating mechanism for the biosorption of Basic violet 3. The results revealed that the yeast *C.tropicalis* has the potential to be used as good adsorbent for the removal of textile dye.

KEYWORDS : *C.tropicalis*; Basic Violet 3; Biosorption; Isotherm models; Adsorption Kinetics.

INTRODUCTION

Synthetic dyes are extensively used in textile, paper, printing industries and dye houses. It is reported that there are over 100,000 commercially available dyes with a production of over 7 x 10^5 metric tonnes per year (Clarke et al., 1980: Zollinger, 1987). Dyeing industry effluents constitute one of the most problematic wastewaters to be treated not for their chemical and biological oxygen demands, suspended solids and content in toxic compounds but also for color, which is the first contaminant to be recognized by human eye. Dyes may significantly affect the photosynthetic activity in aquatic life due to reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides, etc., in them (Clarke et al., 1980: Zollinger, 1987; Mishra and Tripathy, 1993; Banat et al., 1996; Fu and Viraraghavan, 2001; Robinson et al., 2001). Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade.

Dye laden wastewater is usually treated by physical and chemical processes. These include flocculation, electro-floatation, precipitation, electro-kinetic coagulation, ion exchange, membrane filtration, electrochemical destruction, irradiation, ozonation. However, these processes are costly and cannot effectively be used to treat the wide range of dye waste water (Mall et al., 2005). Adsorption has found to be superior to other techniques for wastewater treatment in terms of initial cost, simplicity of design, and ease of operation and insensitive to toxic substances. Activated carbon is the most widely used adsorbent for the removal of dyes and treatment of textile effluents, but it is expensive (Gulnaz et al., 2004). Biological based adsorption (biosorption) uses low cost biological materials viz., living or dead microorganisms for removal of color from wastewaters (Tsezos and Bell, 1989; Fu and Viraraghavan, 2001; Aksu, 2005; Ncibi et al., 2007). However, there are few reports on biosorption of synthetic dyes by yeasts (Farah et al., 2007; Kumari and Abraham, 2007).

In this paper, we present the data on biosorption of synthetic dye Basic Violet 3 by the yeast *Candida tropicalis* isolated from textile wastewater generated in Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India. The influence of some system variables including particle size, pH, biosorbent dosage, initial dye concentration on biosorption were studied. Three adsorption isotherm models viz. Langmuir, Freundlich and Temkin were applied to the experimental data to describe the biosorption equilibrium between dyes and the yeast biomass. The experimental data were also analyzed using the pseudo-first order, pseudo-second order kinetic models. In addition intra particle diffusion model was also performed to evaluate the diffusion mechanism.

MATERIALS AND METHODS

Dye

The synthetic dye Basic Violet 3 was obtained from Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India (**Fig. 1**). The dye stock solution of 1 g/l was prepared in double distilled water. Experimental solutions of desired concentrations were prepared by successive dilutions. The pH of the dye solutions were adjusted using 0.1N HCl and 0.1N NaOH solutions.

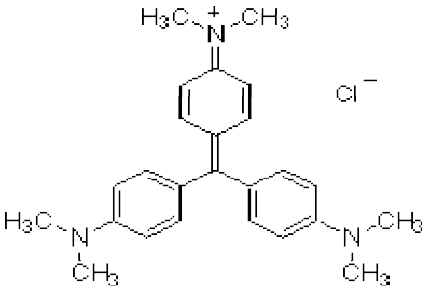

Fig. 1 Structure of Basic Violet 3.

Preparation of Biosorbent

Candida tropicalis was isolated from effluent generated in Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India. The yeast was phenotypically characterized and identified to the species level by Vitek 2 Compact Yeast card reader with the software version V2C 1.01 from Council for Food Research and Development (CFRD), Kerala, India. The isolate was maintained in Yeast Extract Peptone Dextrose (YEPD) agar slants at 4°C. The aqueous extract of sugarcane bagasse was chosen as growth medium for yeast and prepared as reported in our previous study (Das et al. 2010). The medium was adjusted to pH 5.0 with 0.1N HCl and 0.1N sodium hydroxide solutions. Mass cultivation of yeast *Candida tropicalis* was carried out in cost effective sugarcane bagasse extract medium. The yeast biomass was harvested by centrifugation at 8000 rpm for 10 min and subjected to drying at 60°C until a constant weight of biomass was obtained. For biosorption studies, the dried yeast biomass was then finely powdered and sieved through standard sieves to constant sizes.

Batch experiments

The batch sorption experiments were conducted in 250 ml Erlenmeyer flask containing 100ml of working solutions. The solutions were agitated for desired time at 120 rpm and the samples were withdrawn at desired time and subjected to centrifugation at 10,000 rpm for 5 min. The residual dye concentrations of the supernatants were determined by UV-Visible spectrophotometer. Negative controls (with no sorbent) were carried out to ensure that sorption was only by dried biomass of yeast *C.tropicalis* and sorption effect of dye onto the wall of conical flasks were ruled out. All the experiments were performed in triplicates. The dye removal efficiency of yeast biosorbent was calculated as Eq. (1):

$$\text{Dye removal efficiency} = \frac{C_0 - C}{C_0} \times 100 \qquad (1)$$

Where C_0 and C are the concentrations (mg/l) of the dye in the initial solution and after biosorption respectively. The amount of dye adsorbed onto the yeast biomass was obtained by using the following expression Eq. (2),

$$q = \frac{C_0 - C}{M} \times V \qquad (2)$$

Where q is the sorption capacity i.e. the amount of dye biosorbed onto the unit amount of biomass (mg/g); C_0 and C are the concentrations (mg/l) of the each dye in the initial solution and after biosorption respectively; V is the volume of the aqueous phase (l); and M is the amount of the biomass (g).

Influence of operational parameters on biosorption

The influence of particle size on dye biosorption was examined with particle sizes of three ranges: 150-300 µm, 300-425 µm and 425-600 µm. To study the effects of pH, experiments were conducted varying the pH from 2.0 to 8.0. The influence of sorbent dosage was studied with biosorbent concentration varying from 1 to 9 g/l. The influence of initial dye concentration was studied by varying the dye concentrations from 10-120 mg/l with optimum pH values.

Adsorption and Kinetic models

The adsorption and kinetic studies were performed by varying the initial dye concentration from 10- 120 mg/l with biosorbent dosage of 3g/l. After equilibration, the samples were separated and analysed for their dye concentration using UV visible spectrophotometer. From the experimental data, the applicability of Langmuir, Freundlich and Temkin isotherm models were judged. R^2 (regression coefficient square value) and isotherm constant values were determined from these models. The adsorption kinetic data of dyes were analyzed using pseudo first order pseudo second order and intraparticle diffusion equations.

RESULTS AND DISCUSSION

Influence of particle size on biosorption

The influence of particle size on biosorption was analyzed by varying the biosorbent particle size in the ranges 150-300 µm, 300-425 µm and 425-600 µm in 10 mg/l of dye solution with biosorbent dosage of 1g/l. Smallest particle size (150-300 µm) provided larger surface area and resulted in higher adsorption capacity (**Fig 2**). The largest particle size (425-600 µm) resulted in low adsorption capacity. Though smallest particle size exhibited best adsorption capacity, larger particle size was selected for the further study because larger particles can withstand extreme conditions employed in regeneration process (Volesky, 2001).

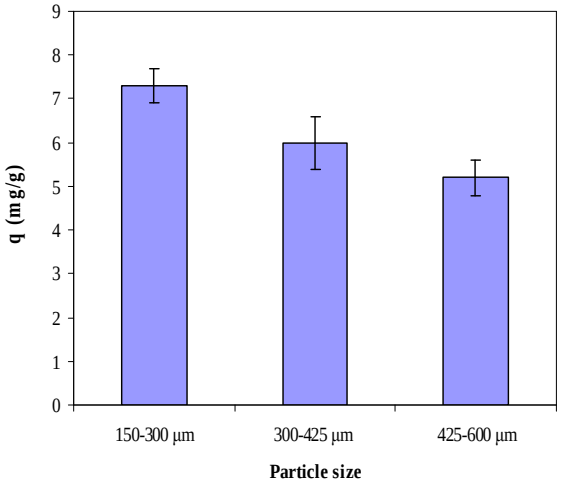

Fig. 2 Influence of particle size on biosorption of Basic Violet 3 by *C.tropicalis*. Initial dye concentration: 10 mg/L; pH: 5; Biosorbent dosage: 1 g/1.

Influence of pH on biosorption

The influence of pH on the amount of biosorption was analyzed in dye solution of concentration 10 mg/l over the pH range from 2 – 8 with 1g/l of biosorbent. As shown in **Fig. 3,** the maximum biosorption capacity was observed at pH 5. The results suggested that the pH of the solution affected the surface charge of the adsorbents as well as the degree of ionization of the dye molecules present in the solution. On increasing the initial pH of the test solution, the net negative charge on the biosorbent surface increased, which favoured the adsorption of positively charged dye molecules (Basic Violet 3) on to the biosorbent surface due to electrostatic attraction.

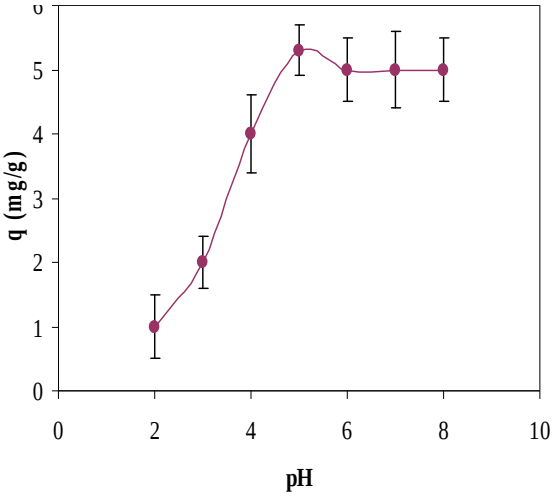

Fig. 3 Influence of pH on biosorption of Basic Violet 3 by *C.tropicalis*. Initial dye concentration: 10 mg/L; pH: 2-8; Biosorbent dosage: 1 g/l.

Influence of biosorbent dosage on biosorption

The experiment on the influence of biosorbent dosage on biosorption of synthetic dye was carried out with different dosage (1, 2, 3, 4, 5, 6, 7g/l). It is readily understood that the number of available biosorption sites increased with an increase in biosorbent dosage and resulted in increased biosorption **(Fig. 4).**

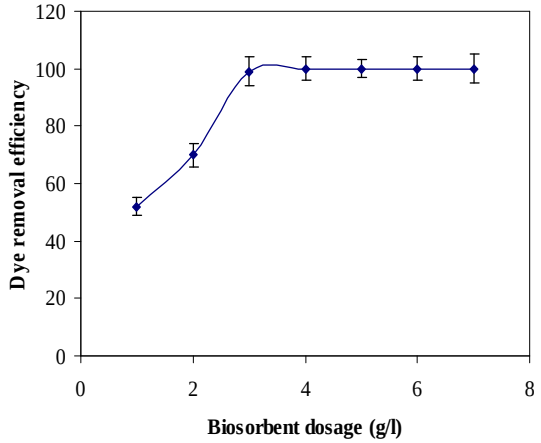

Fig. 4 Influence of Biosorbent dosage on dye removal % for Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 1-7 g/l; Initial dye concentration: 10 mg/l; pH: 5.

As shown in **Fig. 5,** the decrease in sorption capacity may be due to the decrease in solute transfer rate onto the adsorbent surface, i.e., the amount of solute adsorbed onto unit weight of adsorbent gets split with increasing biomass concentration (Vasanth et al. 2005).

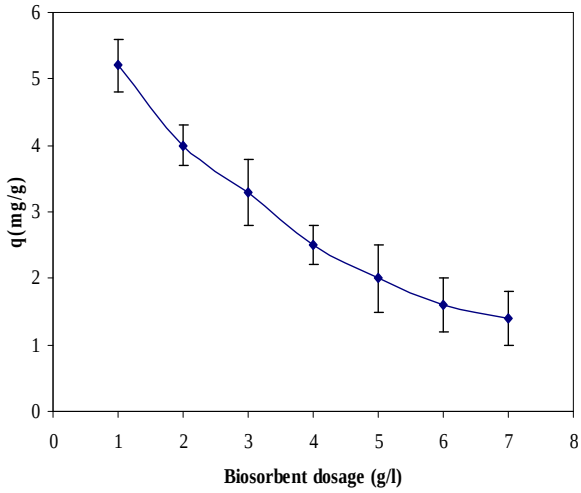

Fig. 5 Influence of Biosorbent dosage on sorption capacity (q) for Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 1-7 g/L; Initial dye concentration: 10 mg/L; pH: 5.

Influence of initial dye concentration on biosorption

The influence of initial concentration of dyes on biosorption was investigated by varying the dye concentrations from 10-120 (mg/l). The sorption capacity of the biosorbent increased with increase in the initial concentration of dye (**Fig. 6**). Initial concentration provides an important driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases. Hence a higher initial concentration of dye may enhance the adsorption process (Aksu, 2005).

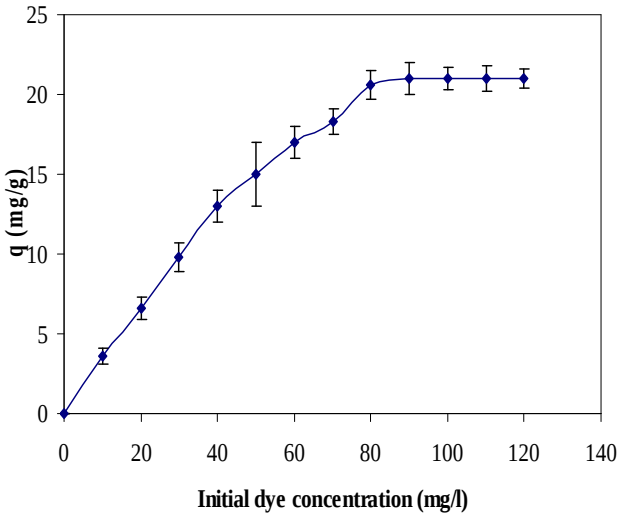

Fig. 6 Influence of initial dye concentration on biosorption of Basic violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-120 mg/l; pH: 5.

Equilibrium Isotherms

Isotherms are the equilibrium relations between the concentration of the adsorbent on the solid phase and its concentration in the liquid phase. From the isotherms the maximum adsorption capacity (q_{max}) can be obtained. Analysis of such isotherms is important in order to develop an equation which accurately represents the results and could be used for designing purposes. Langmuir, Freundlich and Temkin models are the most common isotherms describing solid-liquid sorption systems.

Langmuir isotherm (Langmuir, 1916) makes assumptions, such as monolayer sorption onto a surface with a finite number of identical sites and is given by the following Eq. (3):

$$q_{eq} = \frac{q_{\max} b C_{eq}}{1 + b C_{eq}} \qquad (3)$$

Where, q_{eq} (mg/g) and C_{eq} (mg/l) are amount of adsorbed dye per unit weight of biomass and unadsorbed dye concentration in solution at equilibrium, respectively. q_{max} (mg/g) is the maximum amount of dye per unit weight of biomass required to form a complete monolayer on the surface bound at high C_{eq} and b is a constants, q_{max} and b are evaluated from the linear plot of the logarithmic equation Eq. (4):

$$\frac{1}{q_{eq}} = \frac{1}{q_{\max}} + \frac{1}{b q_{\max} C_{eq}} \qquad (4)$$

The linear plot of $1/q_{eq}$ against $1/C_{eq}$ shows that the adsorption obeys the Langmuir model (**Fig. 7**). Langmuir constants q_{max} and b were determined from the slope and intercept of the plot and presented in **Table 1.** The R^2 value (0.9917) suggested that the Langmuir isotherm provides a good fit to the isotherm data. Similar results were reported by Farah et al. (2007) in biosorption of Astrazone Blue by dried biomass of Baker's yeast.

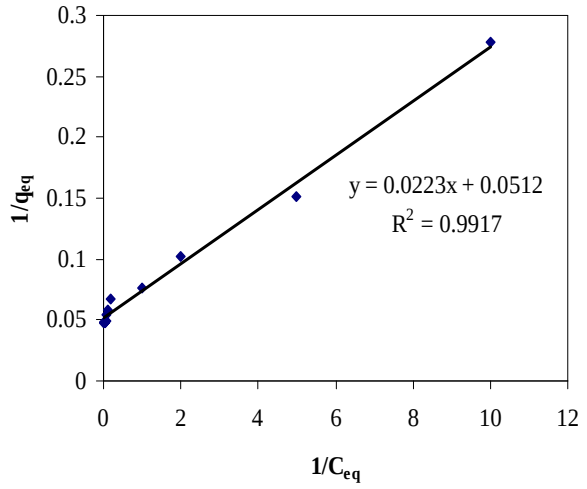

Fig. 7 Langmuir Isotherm for biosorption of Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-120 mg/l; pH: 5.

Table 1 Isotherm constants for Basic Violet 3 on dried biomass of *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-120 mg/l; pH: 5.

Isotherm	Parameters
Langmuir	
q_m (mg/g)	19.53
b	2.2959
R^2	0.9917
Freundlich	
K_f (mg/g) (l/g)$^{1/n}$	9.490
N	4.2140
R^2	0.8899
Temkin	
A (l/g)	59.76
B	2.7229
R^2	0.9739

The Freundlich equation (Freundlich, 1906) describes adsorption onto a heterogenous surface and is given by Eq. (5):

$$q_{eq} = K_F C_{eq}^{1/n} \qquad (5)$$

Where, K_F (mg/g (L/mg)$^{1/n}$ and 1/n are indicators of adsorption capacity and adsorption intensity respectively. In general, as the K_F value increases the adsorption capacity of adsorbent for given adsorbate increases. The magnitude of the exponent, 1/n gives an indication of the favourability of adsorption. Values of n>1 represents favourable adsorption condition (Treybal., 1968; Ho and McKay., 1978). The linear form of equation Eq. (6) is given as:

$$\log q_{eq} = \log K_F + \frac{1}{n} \log C_{eq} \qquad (6)$$

The values of Freundlich constants were calculated from the intercept and slope of the plot (**Fig. 8**) and were listed in Table 1. The R^2 value (0.8899) is lower than Langmuir isotherm. The value of Freundlich exponent n (4.2140) is in the range n>1, indicating a favourable adsorption condition.

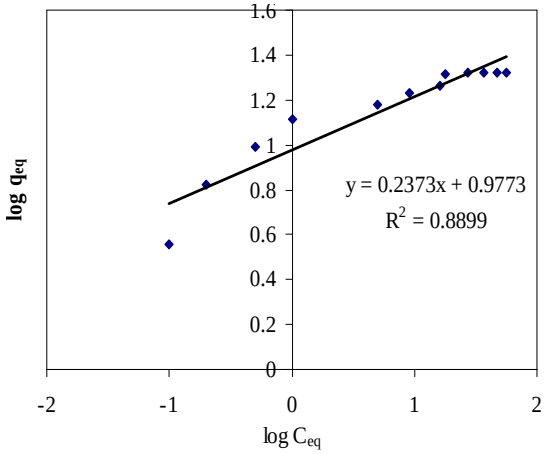

Fig. 8 Freundlich Isotherm for biosorption of Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-120 mg/l; pH: 5.

Temkin isotherm (Temkin and Pyzhev, 1940) assumes that the heat of adsorption of all the molecules in a layer decreases linearly with surface coverage of adsorbent due to sorbate-adsorbate interactions. This adsorption is characterized by a uniform distribution of binding energies. The Temkin isotherm has the following form,

$$q_{eq} = \frac{RT}{b}[\ln(AC_{eq})] \qquad (7)$$

Eq. (7) can be expressed in its linear form as Eq. (8):

$$q_{eq} = B\ln A + B\ln C_{eq} \qquad (8)$$

Where B=RT/b, T is the absolute temperature in K, R the universal gas constant, A the equilibrium binding constant and the constant B is related to the heat of adsorption. According to equation Eq. (8), a plot of q_{eq} versus ln C_{eq} enabled the determination of the isotherm constants A and B (**Fig. 9**) and the values are listed in Table 2. The R^2 value (0.9739) was found to be lower than the Langmuir model.

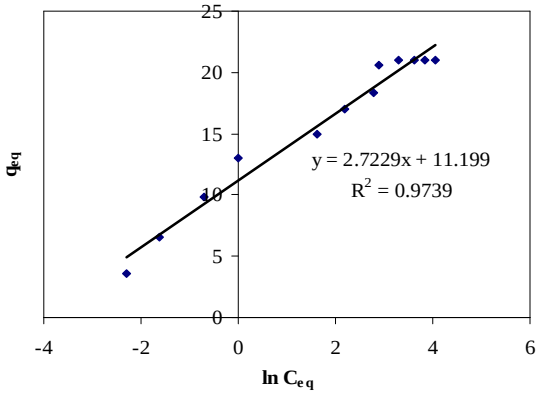

Fig. 9 Temkin Isotherm for biosorption of Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-120 mg/l; pH: 5.

The best equilibrium model was determined based on the linear square regression correlation coefficient R^2. From Table 1, it was observed that the equilibrium data were very best represented by the Langmuir isotherm. This indicates that the adsorption of Basic Violet 3 takes place as monolayer adsorption on yeast biomass surface that is homogenous in adsorption affinity.

Adsorption *Kinetics*

Pseudo-first-order and second-order models were applied to test experimental data and thus elucidated the kinetic adsorption process. Lagergren proposed a method for adsorption analysis which is the pseudo-first-order kinetic equation of Lagergren (Lagergren, 1898) in the form:

$$\log(q_e - q_t) = \log q_e - \frac{K_1}{2.303}t \qquad (9)$$

Where K_1 (1/min), is the rate constant, q_e (mg/g) is the amount of solute adsorbed on the surface at equilibrium and q_t (mg/g) is the amount of solute adsorbed at any time. The value of K_1 was determined from the plot of log(q_e – q_t) against t. The first-order equation of Lagergren (**Fig. 10**) does not fit well to the whole range of contact time and is generally applicable over the initial stage of the adsorption processes.

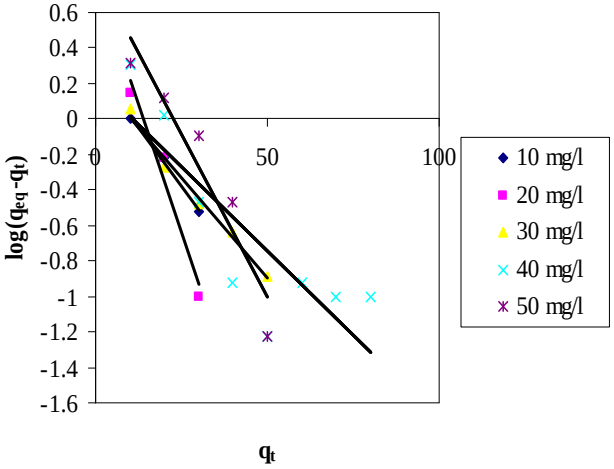

Fig. 10 Pseudo first order kinetics for biosorption of Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-50 mg/l; pH: 5.

Table 2 Comparison of the pseudo-first-order, pseudo-second-order adsorption rate constants and calculated and experimental q_{eq} values obtained at different initial Basic Violet 3 concentrations. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-50 mg/l; pH: 5.

Initial concentration (mg/l)	$q_{eq,exp}$ (mg/g)	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model			
		K_1 (1/min)	$q_{eq,cal}$ (mg/g)	R^2	K_2 (g/mg min) 10^4	$q_{eq,cal}$ (mg/g)	R^2	h_0
10	3.3	0.0601	1.88	0.9924	0.2766	3.3	0.9999	2.7612
20	6.68	0.0131	6.13	0.9591	0.1071	6.68	0.9980	4.7790
30	9.83	0.0515	1.68	0.9847	0.0849	9.94	1.0000	8.3880
40	13	0.0437	1.59	0.7116	0.0533	13.15	0.9999	9.2160
50	15	0.0842	6.71	0.9105	0.0426	15.22	0.9999	9.8580

Although the correlation coefficients (R^2) are generally greater than 0.7116 for all initial concentrations, the parameters indicated that this model had failed to estimate q_{eq} since the experimental values of $q_{eq,exp}$ differs from estimated ones ($q_{eq,cal}$) (Table 2). Thus the adsorption of dyes on the biosorbent cannot be best described by the pseudo-first-orede kinetic. Therefore, the pseudo-second-order-kinetic model (Ho and McKay, 1998) as shown in Eq. (8) was used to study the adsorption kinetic of the present system.

$$\frac{t}{q_t} = \frac{1}{k_2 q_{eq}^2} + \left(\frac{1}{q_{eq}}\right) t \qquad (10)$$

Where k_2 (g/mg min) is the second-order rate constant. The q_{eq} and k_2 can be calculated from the slope and intercept of the plots t/q_t versus t (**Fig. 11**).

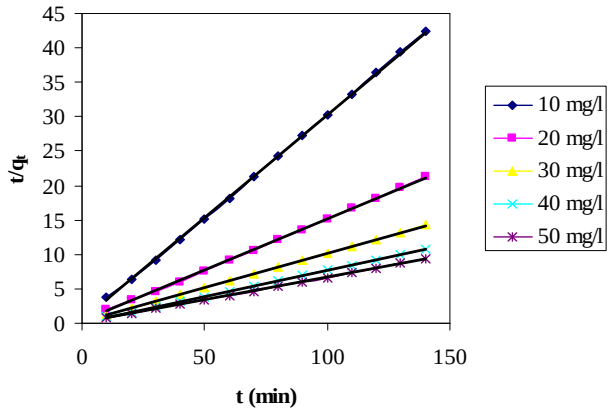

Fig. 11 Pseudo second order kinetics for biosorption of Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-50 mg/l; pH: 5

The constant k_2 is used to calculate the initial sorption rate h (mg/g min), as $t \longrightarrow 0$ as follows:

$$h = k_2 q_{eq}^2 \qquad (11)$$

The pseudo-second-order rate constants k_2, the calculated h values, and the corresponding linear regression correlation coefficients R^2 are given in Table 2. The R^2 values were found to be in the 0.9990-1.000. Moreover, the variations between the calculated ($q_{eq,cal}$) and experimental ($q_{eq,exp}$) were minimal for this model. The high correlations coefficient and high agreement that existed between the calculated and experimental q_{eq} values of the pseudo-second-order kinetics over the other model rendered its best in adsorption of Basic Violet 3 on the yeast biomass. This confirmed that the sorption data were represented by pseudo-second order kinetics for the entire sorption period suggesting that the adsorption process was controlled by chemisorption. The increase in values of the initial adsorption rates, h (Table 3) with an increase in the initial dye concentration could be attributed to the increase in the driving force for mass transfer, allowing more dye molecules to reach the surface of the adsorbents in a shorter period of time (Ho and McKay, 1998).

Table 3 Intraparticle diffusion model constants and correlation coefficients for adsorption of Basic Violet 3 on dried biomass of *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-50 mg/l; pH: 5.

Initial dye concentration (mg/l)	**K_{id} (mg/g $min^{1/2}$)**	**C**	**R^2**
10	0.2728	1.7739	0.9682
20	0.3668	4.2353	0.8803
30	0.2463	8.0541	0.9095
40	0.5221	9.5100	0.9252
50	0.5155	11.3510	0.9971
Average (K_{id})	0.37738		

As the above kinetic models were not able to identify the diffusion mechanism, the kinetic results were further analyzed by intraparticle diffusion model to elucidate the diffusion mechanism (Weber and Mooris, 1963) was tested. It is an empirically found functional relationship, common to the most adsorption processes, where uptake varies almost proportionally with $t^{1/2}$ rather than with in the contact time t. According to this theory,

$$q_t = k_{id} t^{1/2} + C \qquad (10)$$

Where K_{id} (mg.g $min^{1/2}$) is the intraparticle diffusion rate, which can be evaluated from the slope of the linear plot q_t versus $t^{1/2}$. If the regression of qt versus $t^{1/2}$ is linear and passes through the origin, then the intraparticle diffusion is the sole rate limiting step (Poots et al., 1976). For intraparticle diffusion plots, the first, sharper region is the instantaneous adsorption or external surface adsorption. The second region is the gradual adsorption stage where intraparticle diffusion is rate limiting. In some cases, third region exists, which is the final equilibrium stage where intraparticle diffusion starts to slow down due to extremely low adsorbate concentrations left in the solutions (Cheung *et al.*, 2007). As shown in Fig. 12, the plots were not linear over the whole time range, indicating that surface adsorption and intraparticel diffusion were concurrently operating during the Basic Violet 3 biosorption on *C.tropicalis*.

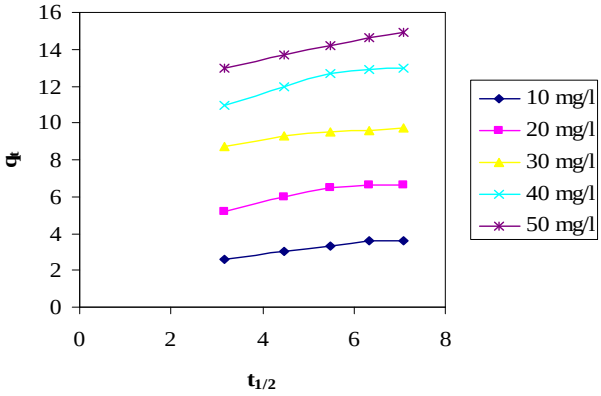

Fig. 12 Intraparticle diffusion model for biosorption of Basic Violet 3 by *C.tropicalis*. Biosorbent dosage: 3 g/l; Initial dye concentration: 10-50 mg/l; pH: 5.

CONCLUSIONS

The results revealed that the potential of dried biomass of *C.tropicalis* for removing Basic Violet 3 from aqueous solutions. Equilibrium data were fitted to Langmuir, Freundlich and Temkin models, and the equilibrium data were best described by Langmuir isotherm with monolayer adsorption capacity of 19.53 mg/g. Kinetic data were tested using pseudo-first order and pseudo second order kinetic models. The kinetics of adsorption process was found to follow the pseudo-second-order model suggesting the adsorption process was controlled by chemisorption process. The plots of intraparticle diffusion model were not linear over the whole time range, implying that more than one process affected the adsorption. The yeast biomass *C.tropicalis* used in this study can be grown in cost effective aqueous extract of sugarcane bagasse. Therefore, the adsorbent is expected to be economically feasible for removal of Basic Violet 3 from aqueous solutions.

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