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Research article

Panus tigrinus as a potential biomass source for Reactive Blue decolorization: Isotherm and kinetic study

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ABSTRACT

Background: Textile and dye industries pose a serious threat to the environment. Conventional methods used for dye treatment are generally not always effective and environmentally friendly. This drove attention of scores of researchers to investigate alternative methods for the biodegradation of dyes using fungal strains. In this work, white-rot fungus (*Panus tigrinus*) was used as a biosorbent for the decolorization of Reactive Blue 19. The process parameters that were varied were initial concentration (50–150 mg/L), contact time (30–90 min), and pH (2–6). In addition, to gain important data for the evaluation of a sorption process, the equilibrium and kinetics of the process were determined.

Results: White-rot fungus showed great potential in decolorizing Azo dyes. The strain showed the maximum decolorization of 83.18% at pH 2, a contact time of 90 min, and an initial concentration of 50 mg/L. The Langmuir isotherm described the uptake of the Reactive Blue 19 dye better than the Freundlich isotherm. Analysis of the kinetic data showed that the dye uptake process followed the pseudo second-order rate expression.

Conclusion: The biosorption process provided vital information on the process parameters required to obtain the optimum level of dye removal. The isotherm study indicated the homogeneous distribution of active sites on the biomass surface, and the kinetic study suggested that chemisorption is the rate-limiting step that controlled the biosorption process. According to the obtained results, *P. tigrinus* biomass can be used effectively to decolorize textile dyes and tackle the pollution problems in the environment.

1. Introduction

Reactive Blue 19 (RB 19) is a textile dye that is easily available and most commonly used in industries such as paper, textile, plastic, carpet and cosmetic industries and processes such as printing, tanning of leather, and processing food because of its suitable properties, i.e., high stability and simple dyeing procedures [1]. Recent research suggests that mutagenic properties are observed in RB 19 because it contains electrophilic vinyl sulfone groups [1,2]. Moreover, RB 19 is highly soluble in water and has a low degree of fixation, resulting in highly colored wastewater. It is designed to convey high photolytic stability and resistance toward major oxidizing agents. It has the ability to form "forbidden aromatic amines," which are toxic and potential carcinogens [3]. Therefore, it is necessary to find an efficient and effective way for RB 19 treatment and decolorization [4].

Currently, a wide range of physical and chemical methods are already in use. However, the processes adopted are not considered as the most practical or sustainable methods for the decolorization of contaminated wastewater [5].

Among all treatment strategies used before, the use of adsorbents in the removal process of reactive dyes from wastewater was shown to be an effective method because of their fast and convenient dye elimination reaction, accompanied with their resistance to toxic contaminants. In addition to their low initial cost, adsorbents have a simple design and operation and nontoxic by-products [6,7]. Moreover, the adsorbent's nature and type have a considerable effect on the adsorption performance [8].

White-rot fungus has been proven to be a suitable organism for treating textile effluent and for dye removal [9]. It uses extracellular and highly nonspecific ligninolytic enzymes (i.e., laccase, manganese peroxidase, and lignin peroxidase). These enzymes degrade lignin and structurally related compounds such as complex aromatic pollutants, which can catalyze different kinds of oxidation and reduction reactions. Moreover, they can form highly reactive oxygen species.

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Chagas and Durrant [10] have reported that fungi have an additional advantage over single-cell organisms as they can solubilize the insoluble substrates.

The mechanisms involved in these studies can be categorized into biosorption, bioaccumulation, and biodegradation [9,11]. In this study, the biosorption mechanism was adopted for the treatment using dead cells (inviable biomass), whereby physiochemical interactions (i.e., adsorption, deposition, and ion exchange) were used.

The aim of this research was to study the optimum conditions for the decolorization of RB 19 using *Panus tigrinus* biomass at pH 4–6, a contact time of 30–90 min, and an initial concentration of 50–150 mg/L. The study also aims to investigate and determine the biosorption kinetics and adsorption isotherms.

2. Materials and methods

The *P. tigrinus* strain used in this work was from the Department of Biotechnology Engineering, IIUM, Malaysia. The cells were preserved by continuous plating on malt extract agar at 32°C for 1 week. Cassava peel, used as the substrate, was collected from a small-scale kerepek (a type of local snack) industry in Kuala Langat, Selangor, Malaysia. The cassava peel was washed properly to remove sand and tuber head. Then it was immediately dried at 60°C in an oven for 48 h to prevent the growth of unwanted microbes. The dried peels were then ground and sieved to 1-mm particle size [12]. RB 19 was manufactured by Sigma Aldrich and supplied by Bumi-Pharma Sdn Bhd (Table 1). The stock solution was prepared using the method by Jamal et al. [12].

2.1. Preparation of inoculum and production of biomass

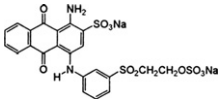
Four batches of fungal culture were washed with 15 mL of distilled water. The spores were collected in an Erlenmeyer flask by gentle scrapping using sterile glass rod.

For the production of the biosorbent, cassava peel (5.14 g) and wheat flour (0.68 g) were used as carbon sources for fermentation. Then 11.8 mL water and 1 mL of mineral solution [$(NH_4)_2SO_4$ 1.5 g, $MgSO_4$ 0.45 g, $MnSO_4$ 0.5 g, and KH_2PO_4 0.8 g) were added, and the mixture autoclaved at 121°C for 15 min. Subsequently, 1.2 mL of the inoculum was added to the sample, and the samples were incubated for 6 d at 32°C. The samples were centrifuged at 8500 rpm with an equal amount of hexane to remove the excessive oil residues, and the extraction process was performed using chloroform:methanol. The fungal biomass was removed and stored for further experiments.

2.2. Biosorption of RB 19

In this study, three factors were investigated for the removal of dye, namely the initial concentration (50, 75, 100, 125, and 150 mg/L), contact time (30, 45, 60, 75, and 90 min), and pH (2, 3, 4, 5, and 6). The specified values for time, pH, and initial concentration were selected according to data obtained from previous studies. The response tested was the percentage decolorization.

Table 1
Reactive Blue 19 properties.

Name	C.A.S.	F.W.	UV absorption	Molecular structure
Reactive Blue 19	2580-78-1	626.54	$\lambda max = 596$ nm	

The amount of dye adsorbed by the *P. tigrinus* biomass at the equilibrium time was calculated using [Equation 1]:

$$q_e = \frac{(C_i - C_f)}{W} V \qquad \text{[Equation 1]}$$

Where q_e is the amount of dye adsorbed per gram of biosorbent at equilibrium (mg/g), C_i (mg/L) is the initial dye concentration, C_f (mg/L) is the final dye concentration, V (L) is the volume of dye solution in the flasks, and W (g) is the mass of used biosorbent [13].

The percentage removal of dye is defined as the ratio of difference in dye concentration before and after adsorption to the initial concentration of dye in the aqueous solution and was obtained by using [Equation 2]:

$$\text{Removal } percentage = \frac{(C_i - C_f)}{C_i} \times 100 \qquad \text{[Equation 2]}$$

2.3. Adsorption isotherms

Erlenmeyer flasks (50 mL) were used to conduct the batch-system reactions, containing 25 mL of varied concentrations of dye solution (20–100 mg/L). A fixed amount of biosorbent (15 g/L) was poured into the solution and incubated for 1 h at room temperature (26°C) and 200 rpm in an incubator shaker. These incubated samples were withdrawn from the flask to analyze the residual concentration of the dye solution by UV–vis spectrophotometry at the maximum wavelength, 596 nm, for the dye. To obtain the numerical estimate of the adsorbent capacity, Freundlich and Langmuir adsorption isotherms were used for this system [17].

2.4. Kinetics study

Kinetics is of great importance to evaluate the performance of a given adsorbent and gain insight into the underlying mechanisms [14]. The nature of the process depends on chemical or physical characteristics of the adsorbent system and also on the system conditions [15]. The adsorption rate can be established quantitatively by the pseudo-first-order and pseudo-second-order models [16].

The kinetics of the adsorption process can be premeditated by conducting a different set of adsorption experiments at constant temperature (26°C) and a variable time (10–60 min). For this experiment, 15 g/L of biosorbent was added into an Erlenmeyer flask containing 25 mL of 50 mg/L dye solution. The flask was then agitated at 200 rpm, and the samples were collected at appropriate time intervals. The samples were analyzed by UV–vis spectrophotometry.

3. Results and discussion

3.1. Influence of contact time

Dye removal time is a crucial parameter to be considered as it can determine the energy and cost of the removal process. Longer time taken to remove the dye increases the cost of energy to be supplied. Xiong et al. [18] studied the removal of CI Direct Blue 199 from aqueous solution by nonviable *Aspergillus niger* and concluded that the biosorption process reached equilibrium after 40 min, whereas Chander and Arora [19] evaluated some white-rot fungi for their potential to decolorize industrial dyes and established that a large part of decolorization occurred at the first 2–5 h.

The effect of time on dye removal in the present study is shown in Fig. 1. The dye removal process commenced at 30 min, and it gradually increased until it reached a maximum of 30% at 60 min where the process reached equilibrium and then decreased slightly with the passing of time. This slight decrease in dye removal can be

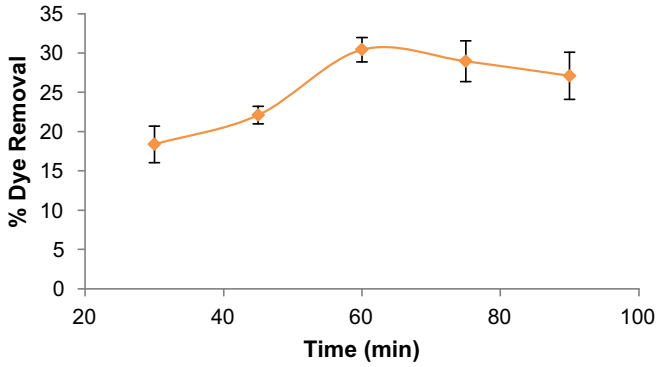


Fig. 1. Effect of contact time (min) on dye removal (%).

explained by the fact that the biomass was completely saturated with the dye when the time duration exceeded 60 min. This finding is in line with the results of Brahimi-Horn's study where it was concluded that in the case of nonviable cells, a duration of 1 h can be sufficient to remove large quantities of the dye [19]. Therefore, we considered 1 h as the optimum period for the adsorption of RB 19.

3.2. Influence of initial concentration

The dye concentration in textile process wastewater is in the range of 10–200 mg/L [20], and therefore, the initial dye concentration in this research varied within the same range. The initial dye concentration was an important driving force to overcome all mass transfer resistance of dye between the aqueous and solid phases [21,22]. The effect of initial concentration can be seen in Fig. 2. At 50 mg/L, the percentage of dye removal was 25.71%. The percentage of dye removal increased slightly with an increase in concentration until it reached the concentration of 150 mg/L (29.20%).

The results obtained in this section were not in agreement with those of Mona et al. [13], who observed that an increase in the dye concentration decreased the dye removal and that this decline was due to some inhibitory effects of dye toxicity at higher concentrations. Reduced dye removal at higher concentration has also been stated by other researchers for the removal of basic yellow dye, reactive red 120, and other reactive dyes through microbial biosorption [13,21,23]. Moreover, the percentage of dye removal decreased from 97 to 75% when *Rhizopus stolonifer* was used for bromophenol blue [22]. In the present study, no decline in the dye removal was observed, indicating that no inhibitory effects were present within the selected time interval.

3.3. Influence of pH

pH is the most important parameter affecting the biosorption capacity, color of the dye solution, and the solubility of some dyes [24]. This is because it influences the chemistry of dye molecules and fungal biomass in an aqueous solution. The pH of the solution influences the surface charge of the adsorbent, which in turn influences the adsorption of charged dye groups. This is because negatively charged dye anions bind to the positively charged biomass. Therefore, in the case of acidic dyes, the biosorption capacity increases when the pH of the solution is low, and the opposite is observed for alkaline conditions [9,24,25].

It was reported that *Rhizopus arrhizus* and *Phanerochaete chrysosporium* showed higher biosorption capacities for the acidic dyes Remazol Blue and Remazol Brilliant Blue R at pH 2 than at pH 10 [26,27]. In another investigation of the effect of initial pH on the ability of dried *R. arrhizus* for the removal of Reactive Red, Remazol Black B, RB 19, Reactive Orange 16, and Congo Red, the maximum uptake was found to be at pH 2 [24,26,28]. Furthermore, the above stated results were confirmed when studying *Pseudomonas luteola*, *Escherichia coli*, *Aeromonas* biomass, and *Aeromonas* sp. [24] where the best results were obtained at pH 3 [25]. However, it was observed that the sorption of basic methylene blue by *Fomes fomentarius* and *Phellinus igniarius* increased as the pH was increased from 3 to 11 [9,27].

This study agrees with all the previously mentioned work, where it was clear that the effect of pH on the dye removal process was very significant, as illustrated in Fig. 3. The percent dye removal was maximum (82.43%) at pH 2 and minimum (23.17%) at pH 4. Obtaining low levels of removal at pH 4 can be explained by the occurrence of electrostatic repulsion between the fungi and dye as they both have the same pH. High removal percentage at pH 2 was a result of electrostatic attraction between the dye molecules and surface of the biomass. However, some removal of dye (34.81%) was observed at pH 6 because of the weak attraction between the two surfaces. The results obtained here are in agreement with those of previous studies where the researchers could remove 95 mg/g and 53% of RB at pH 2 compared to less than 30 mg/g at pH 10 [9,26,27].

3.4. Isotherms

In the present study, the Langmuir and Freundlich isotherms were used to explain the equilibrium adsorption of RB 19 onto fungal biomass by varying the initial concentration (20, 40, 60, 80, and 100 mg/L). Fig. 4 and Fig. 5 illustrate the Langmuir and Freundlich plots for the biosorption study. The Freundlich constants are linked to the adsorption capacity and adsorption intensity, whereas the Langmuir constants are related to the maximum adsorption capacity and bonding energy of adsorption [29].

From the values of correlation coefficients, R^2, it can be concluded that the experimental data for the biosorption of RB onto biomass was best described by the Langmuir isotherm model. The correlation coefficient, R^2, of the Langmuir isotherm model was greater than that of the Freundlich isotherm model, indicating better fit by the former. However, the values of R^2 for both models are fairly close to each

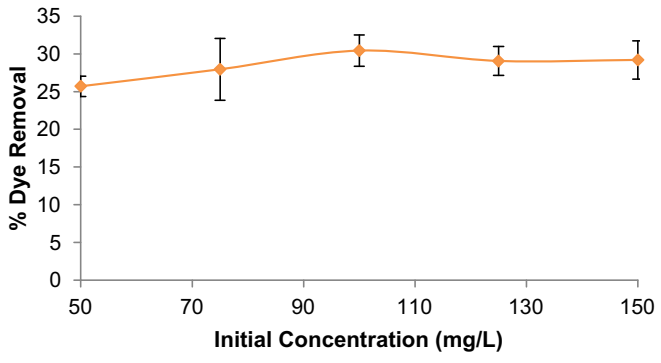


Fig. 2. Effect of initial concentration (mg/L) on percent dye removal.

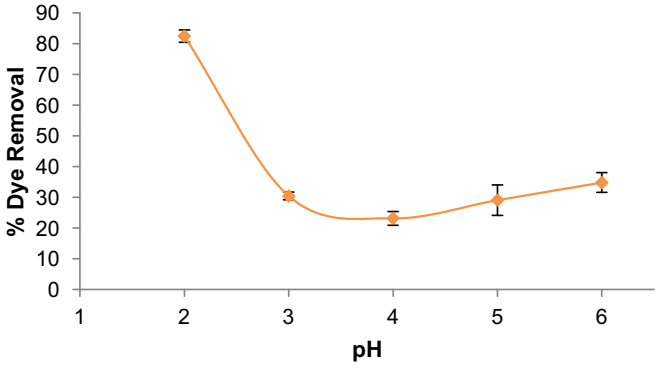


Fig. 3. Effect of pH on percent dye removal.

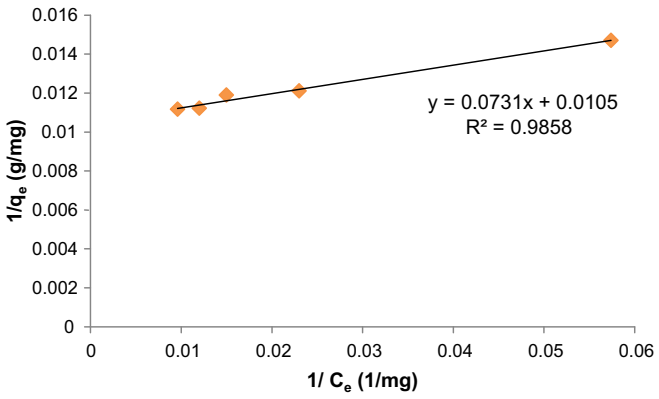


Fig. 4. Langmuir plot for Reactive Blue biosorption onto fungal biomass at different concentrations (Conditions: dosage of 15 g/L, room temperature, contact time of 1 h).

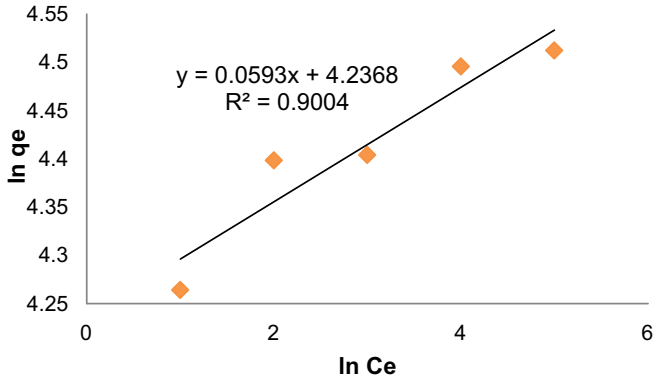


Fig. 5. Freundlich plot for Reactive Blue biosorption onto fungal biomass at different concentrations (Conditions: dosage of 15 g/L, room temperature, contact time of 1 h).

other. According to this model, the monolayer adsorption capacity was 0.0731 g/mg at room temperature. Because the Langmuir isotherm fits well, it can be suggested that there is a homogeneous distribution of active sites on the biomass surface [30]. Zeroual et al. [31] reported that equilibrium data for bromophenol blue using *R. stolonifer* fitted the Langmuir isotherm very well and obtained an R^2 of 0.99 [9,31].

3.5. Kinetics

Fig. 6 and Fig. 7 show pseudo-first-order and pseudo-second-order kinetic plots, respectively. The kinetic parameters for both plots were determined by linear regressions of the proposed models following

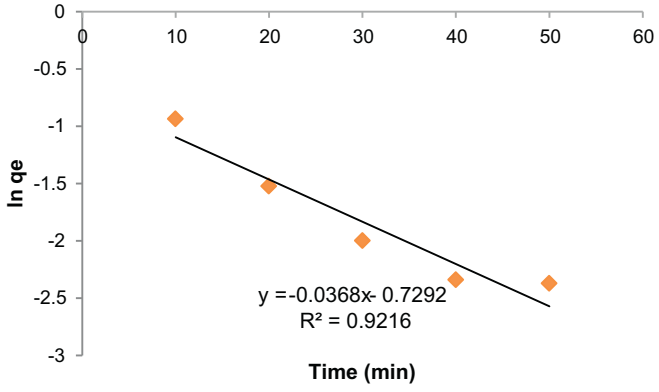


Fig. 6. Pseudo-first-order kinetics for the biosorption of Reactive Blue onto 15 g/L fungal biomass at room temperature and an initial concentration of 100 mg/L.

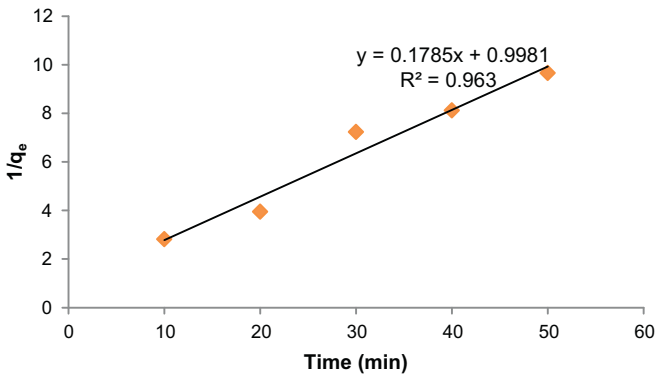


Fig. 7. Pseudo-second-order kinetics for the biosorption of Reactive Blue onto 15 g/L fungal biomass at room temperature and an initial concentration of 100 mg/L.

the linear equation below:

$$\log(q_e\text{-}q_t) = \log q_e\text{-}\frac{K_f}{2.303}t \qquad \text{[Equation 3]}$$

where q_e and q_t are the amounts of dye adsorbed (mg/g) at equilibrium and at time t (min), respectively, and K_f is the rate constant of adsorption (min^{-1}) [18,32]. The linear form of the pseudo-second-order equation is given by [Equation 4]:

$$\frac{t}{q_{(t)}} = \frac{1}{K_F q_e^2} + \frac{1}{q_e}t \qquad \text{[Equation 4]}$$

With this equation, there is no problem in assigning an effective q_e because q_e and K_f can be obtained from the slope and intercept of the plot of t/q_e vs. t, respectively [18,32].

When looking at the correlation coefficients, R^2, for both the models, it can be concluded that the biosorption of RB onto the fungal biomass was best described by the pseudo-second-order model as it had an R^2 of 0.9632, whereas the pseudo-first-order model had an R^2 of 0.9216. It is assumed that the rate-limiting step was the involvement of valence forces by sharing or exchanging electrons between biosorbent and biosorbate, thus providing the best correlation of data [33].

Aksu and Karabayır [34] reported that the time required to reach a state of equilibrium for the biosorption of reactive dyes by the nonviable biomasses of *R. arrhizus*, *Trametes versicolor*, and *A. niger* was approximately 4 h. In addition, Akar et al. [35] suggested that a pseudo-second-order kinetic model is suitable for the removal of Acid Red 44 dye from aqueous solutions by *Agaricus bisporus*, and the equilibrium time was 2 h. Aksu and Tezer [24] applied the pseudo-first- and second-order kinetic models to study Remazol Black B biosorption on dried *R. arrhizus*, and their results indicated that the dye uptake process followed the pseudo-second-order rate expression. Moreover, Abdul Rahim and Garba [36] used activated carbon for the removal of 4-chloroguiacol from aqueous solution, and their results revealed pseudo-second-order to be the most ideal model for describing the kinetic data. Furthermore, Bhagya et al. studied the removal of hazardous dyes from aqueous solutions using $SrTiO_3$ nanoparticles, where the best representation of the data was by the Langmuir isotherm model [37].

4. Conclusion

In this study, the effect of *P. tigrinus* biomass on RB 19 dye was investigated. The highest dye removal was observed at pH 2, contact time of 90 min, and an initial concentration of 50 mg/L (83.18%), whereas the lowest was observed at pH 6, contact time of 30 min, and an initial concentration of 50 mg/L (13.18%).

From the isotherm study, it can be noted that the experimental data better fitted the Langmuir isotherm model for the biosorption of RB onto biomass than the Freundlich isotherm. However, the results of the kinetic study indicated that the biosorption of the selected dye followed the pseudo-second-order kinetic model, suggesting that chemisorption might be the rate-limiting step that controls the biosorption process.

Competing interests

The authors declare that they have no competing interests.

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