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Biosorption of Reactive Blue 4 dye by native and treated fungus *Phanerocheate chrysosporium*: Batch and continuous flow system studies

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Abstract

The native and treated fungal biomass of *Phanerocheate chrysosporium* was used for the biosorption of a textile dye (i.e., Reactive Blue 4). In the batch system, the biosorption equilibrium time was about 4 h and the maximum dye uptake on all the tested fungal biomass preparations was observed at pH 3.0. The dye uptake capacities of the biosorbents at 600 mg L^{-1} dye concentration were found to be 132.5, 156.9, 147.6 and 81.1 mg g^{-1} for native and heat-, acid- and base-treated dry fungal preparations, respectively. The dye uptake capacity order of the fungal preparations was found as heat-treated > acid-treated > native > base-treated. The Langmuir, Freundlich and Temkin adsorption models were used for the mathematical description of the biosorption equilibrium. The Freundlich and Temkin models were able to describe the biosorption equilibrium of Reactive Blue 4 on native and treated fungal preparations. The dye biosorption on the fungal biomass preparations followed Ritchie kinetic model. Biosorption of the dye from aqueous solution was also investigated in a continuous flow system. The maximum biosorption capacity of the heat-treated fungal biomass *P. chrysosporium* was 211.6 mg (g dry biomass)$^{-1}$ at an initial dye concentration of 600 mg L^{-1} and at a flow rate of 20 mL h^{-1}.

Keywords: *Phanerocheate chrysosporium*; Dye biosorption; Textile dye; Reactive Blue 4; Kinetics; Adsorption isotherm model

1. Introduction

The reactive triazine dyes are heavily used in textile industry for coloring different cloth materials and their metabolites (e.g. aromatic amines) can be highly toxic and potentially carcinogenic, mutagenic and allergenic on exposed organisms [1–4]. The dye components are hardly degradable by physicochemical or biological methods. Most of the triazine dyes are also resistant to chemical, photochemical and biological degradation [5–7].

A range of conventional treatment technologies for the removal of textile dyes have been investigated extensively. The used methods are activated sludge, chemical coagulation, carbon adsorption, chemical oxidation, photodecomposition, electrochemical treatment, reverse osmosis, hydrogen peroxide catalysis, etc. [8–11]. However, these methods are found to be ineffectual due to their cost, regeneration or reusability. Biomass of some natural microbial species, including bacteria, fungi and algae, is capable of removing the different textile dyes by biosorption, biodegradation or mineralization [12]. Some low cost fungal biomass has been used as biosorbent for the removal of dye and metal ions from or wastewater, which included *Trametes versicolor* [13], *Corynebacterium glutamicum* [14], *Lentinus sajor caju* [15,16], *Rhizopus nigricans* [17], *Cunningamella polymorpha* [18], *Funalia trogii* [19] and *Aspergillus niger* [7]. The white rot fungus, *Phanerocheate chrysosporium*, have been widely used for bioremediation studies [20–23]. However, a few studies have been focused on utilization of the fungus for dye removal.

In this study, the native and heat-, acid- and alkali-treated biomasses of *P. chrysosporium* were used for the biosorption of Reactive Blue 4 in batch and continuous systems. The effects of experimental conditions such as initial dye concentration, temperature and pH are investigated to obtain information on dye removal properties of the fungal biomass. The possible mechanisms involved in the dye biosorption are discussed on the basis of the treatment of the fungal biomass followed by contact angles and FTIR spectroscopic studies. Finally, acid-treated fungal biomass was operated in a packed bed reactor for removal of the dye from artificial waste streams.

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1.1. Biosorption isotherm models

The Langmuir and Freundlich adsorption isotherm models are widely used to analyse data for water and wastewater treatment applications. These equations can be written in the form given below to predict the adsorption capacities of the biosorbent:

$$Q = \frac{Q_{\max} b C_{\mathrm{eq}}}{1 + bC_{\mathrm{eq}}} \tag{1}$$

$$Q = K_{\mathrm{F}}(C_{\mathrm{eq}})^{1/n} \tag{2}$$

For the Langmuir model (Eq. (1)), the constant b is related to the energy of adsorption, C_{eq} is the equilibrium concentration of the dye in solution, Q the amount of adsorbed dye on the biosorbent surface and the constant $Q_{\max}$ represents the maximum binding sites. The Freundlich adsorption isotherm constants, K_{F} and n, are indicative of the extent of the adsorption and the degree of non-linearity between solution concentration and adsorption, respectively. The Temkin isotherm model assumes that adsorption is characterized by a uniform distribution of binding energies, up to some maximum binding energy ($\Delta G_{\max}$), which results in the following isotherm equation [24]:

$$Q = Q_{\mathrm{T}} \ln(1 + K_{\mathrm{T}} C) \tag{3}$$

where K_{T} ($\mathrm{L\,mol^{-1}}$) is the equilibrium binding constant corresponding to the maximum binding energy ($K_{\mathrm{T}} = \exp(-\Delta G_{\max}/RT)$, and Q_{T} is the differential surface capacity for dye biosorption per unit binding energy.

1.2. Biosorption kinetic modeling

The kinetics of dye biosorption on the fungal biomass preparations was determined with two different kinetic models, i.e., the first-order [25] and Ritchie kinetic model [26]. The first-order rate equation of Lagergren is:

$$\frac{\mathrm{d}q_t}{\mathrm{d}t} = k_1(q_{\mathrm{eq}} - q_t) \tag{4}$$

where k_1 is the rate constant of first-order biosorption ($\mathrm{min^{-1}}$) and q_{eq} and q_t denote the amounts of biosorption at equilibrium and at time t ($\mathrm{mg\,g^{-1}}$), respectively. After integration by applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives:

$$\log\left(\frac{q_{\mathrm{eq}}}{q_{\mathrm{eq}} - q_t}\right) = \frac{k_1 \cdot t}{2.303} \tag{5}$$

The Ritchie kinetic model equation is:

$$\left(\frac{1}{q_t}\right) = \left(\frac{1}{k_2 q_{\mathrm{eq}} t}\right) + \left(\frac{1}{q_{\mathrm{eq}}}\right) \tag{6}$$

where k_2 ($\mathrm{g\,mg^{-1}\,min^{-1}}$) is the rate constant of the Ritchie kinetic model.

A plot of $1/q_t$ versus $1/t$ (Eq. (6)) should give a linear relationship for the applicability of the Ritchie kinetic model.

The intra-particle diffusion model proposed by Weber and Morris [27], the initial rate of intra-particular diffusion is calculated by linearisation of the curve $q = f(t^{0.5})$:

$$q = K_{\mathrm{i}} t^{0.5} \tag{7}$$

where q is the amount of adsorbed dye on the fungal biomass preparations at time t ($\mathrm{mmol\,g^{-1}}$), t the time (s) and K_{i} is the diffusion coefficient in the solid ($\mathrm{mmol\,g^{-1}\,s^{1/2}}$). K_{i} has been determined by a plot $q = f(t^{0.5})$ taking account only of the initial period.

2. Materials and methods

2.1. Materials

Reactive Blue 4 was obtained from Sigma–Aldrich Chemical Co., St. Louis, MO, USA. The chemical structure and some properties of the reactive dye are presented in Fig. 1 and Table 1. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany). The water used in the following experiments was purified using a Barnstead (Dubuque, IA, USA) ROpure LP reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure organic/colloid removal and ion exchange packed-bed system.

2.2. Cultivation of fungus

The white rot fungus "*P. chrysosporium* (ATCC-34541)" was cultivated in liquid medium using the shake flask method. The growth medium consisted of ($\mathrm{g\,L^{-1}}$ of distilled water): D-glucose (10.0); KH_2PO_4 (20.0); $MgSO_4{\cdot}7H_2O$ (0.5); NH_4Cl (0.1); $CaCl_2{\cdot}H_2O$ (0.1); thiamine (0.001). The pH of the medium was adjusted to 4.5 before autoclaving. Once inoculated, flasks were incubated on an orbital shaker at 150 rpm for 7 days at 30 °C. After incubation, the biomass was harvested from the medium and washed with distilled water.

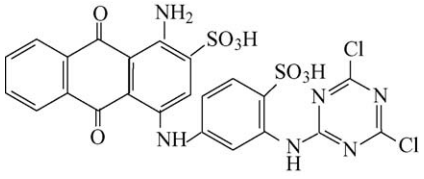

Fig. 1. The chemical structure of the Reactive Blue 4.

Table 1
The general characteristics of Reactive Blue 4

Chemical formula	$C_{22}O_8H_{22}N_6S_2Cl_2$
Molar mass	637.4
C.I. number	61205
$\lambda_{\max}$ (nm)	595

2.3. Preparation of fungal biosorbents

Heat-treated form of *P. chrysosporium* was prepared in physiological saline solution by heating at 100 °C at 10 min and after treatment referred as heat-treated fungal biomass. The native fungal biomass (about 10.0 g) was transferred into 50 mL 0.1 M HCl and/or NaOH solutions and the mixture was stirred at 250 rpm for 1.0 h at ambient temperature and, hereafter they called acid- and/or base-treated fungal biomass. Each treated fungal biomass was centrifuged at 5000 rpm for 10 min, washed with sterile physiological saline buffer solution and dried in a vacuum oven at 50 °C.

2.4. Biosorption studies

The biosorption of Reactive Blue 4 on the native and heat-, acid- and base-treated fungal biomass was investigated in a batch system. A stock solution (1000 mg L^{-1}) of dye was obtained by dissolving the dye in the purified water. The dye solutions with different concentrations were prepared from this stock solution. To determine the effect of initial concentrations of the dye on the biosorption rate and capacity on the fungal preparations, the initial concentration of the dye was varied between 25 and 600 mg L^{-1} in the biosorption medium.

The effects of the medium pH on the biosorption capacities of the fungal biomass preparations were investigated in the initial pH range 3.0–10.0 (which was adjusted with H_2SO_4 or NaOH at the beginning of the experiment and not controlled afterwards) at 25 °C. The effect of temperature was studied at four different temperatures (i.e., 4, 15, 25 and 37 °C) at pH 3.0. In a typical biosorption experiment, fungal biomass (250 mg wet weight) in 10 mL of dye solution was agitated magnetically at 150 rpm for 6 h at 25 °C. The concentration value of the biosorption samples was 200 mg L^{-1}. Before analysis of the remaining dye concentration, the sample was centrifuged at 3000 rpm. The amount of biosorbed dye per unit fungal biomass (mg dye (g dry biomass)$^{-1}$) was obtained by using the following expression:

$$q = \frac{(C_o - C) \cdot V}{M} \tag{8}$$

where q is the amount of dye biosorbed onto the unit amount of the biomass (mg g^{-1}); C_o and C the concentrations of the each dye in the initial solution (mg L^{-1}) and after biosorption, respectively; V the volume of the aqueous phase (L); and M is the amount of the biomass (g). The dissolved dye concentrations of the samples were analysed using a double beam UV/vis spectrophotometer (Shimadzu, Tokyo, Japan; Model 1601) at 595 nm for Reactive Blue 4. Results given in averages were obtained from the experiments repeated three times.

2.5. Continuous system

The continuous system column was made from Pyrex glass (length 8.0 cm, diameter 1.2 cm, total volume about 9.0 mL). The acid-treated fungal biomass (wet weight about 7.0 g) was loaded into the continuous system yielding a void volume of about 2.0 mL. The dye solution at a known concentration and flow rate was introduced to the system. The flow rate was regulated between 20 and 120 mL h^{-1} with a peristaltic pump (ISMATEC, IPC Model) though the lower inlet part in order to avoid channeling of the effluent. The concentration of the dye was kept constant at 400 mg L^{-1} for each individual run. The system was operated at 25 ± 0.2 °C and at pH 3.0 for 10 h. The dye sample leaving the system was collected and the amount of biosorbed dye per unit fungal biomass (q) was calculated according to Eq. (7).

In order to determine the effect of initial dye concentration on the biosorption efficiency, the initial dye concentration was varied between 50 and 600 mg L^{-1} in the feed solution and was pumped through the lower inlet part of the continuous system at a flow rate of 100 mL h^{-1}. The continuous system was operated as described above and the residence time corresponding to the given flow rates is calculated by the following equation:

$$D = \frac{\nu_o}{\varepsilon V} \tag{9}$$

where D is the dilution rate (h^{-1}), ν_o the volumetric flow rate of the dye solution (mL h^{-1}), V the total volume of the continuous system (mL) and ε is the void fraction of given as ratio of void volume to the total volume of the continuous system. Residence time (τ) is the reciprocal of dilution rate.

$$\tau = \frac{1}{D} \tag{10}$$

2.6. Characterization of the fungal biomass

2.6.1. Determination of the water content

The fungal biomass "*P. chrysosporium*" was allowed to soak in saline solution for 24 h, swollen biomass was weighed after removing of the excess water and then was dried in vacuum oven at 60 °C for 24 h until constant weight.

2.6.2. Scanning electron microscopy

Scanning electron micrograph of the nature biomass *P. chrysosporium* was obtained using a JEOL, JMS 5600 scanning electron microscope, after coating with thin layer gold under reduced pressure.

2.6.3. The FTIR spectra

The IR spectra of native, acid-, base- and heat-treated *P. chrysosporium* were obtained by using a FT-IR spectrophotometer (Mattson 1000 FT-IR, England). For FTIR spectra, approximately fungal biomass (0.01 g) was mixed with KBr (0.1 g) and pressed into a tablet form by pressing the ground mixed material with the aid of a bench press. The FTIR spectrum was then recorded.

2.6.4. Contact angle measurement

Contact angles to different test liquids (i.e., water, glycerol and DIM) of all the investigated fungal biomass film preparations were measured by sessile drop method at 25 °C by using a digital optical contact angle meter CAM 200 (KSV Instruments Ltd., Helsinki, Finland). Both the left and right contact angles and drop dimension parameters of the fungal samples

were automatically calculated from the digitalized image using CAM 200 software operated under Windows 98. The measurements were the average of five contact angles at least operated on three fungal biomass film samples.

3. Results and discussion

3.1. Properties of fungal biomass

The surface morphology of the native *P. chrysosporium* mycelia is exemplified by the scanning electron micrograph in Fig. 2. As shown in the SEM micrograph, the fungal mycelia have rough and porous surface. This surface property should be considered as a factor providing an increase in the total surface area.

In order to confirm the existence of functional biosorption groups (i.e., amino, carboxyl and phosphate) on the fungal biomass, the FTIR spectra of fungal biomass preparations were obtained. The spectra for native fungal biomass are exemplified in Fig. 3. In general, the FTIR spectra of all the fungal preparations have intense peaks at a frequency level of 3500–3200 and 1540 cm^{-1} representing amino groups stretching vibrations. The amino groups stretching vibrations bands of fungal preparations are superimposed on the side of the hydroxyl group band at 3500–3300 cm^{-1}. The strong peaks at around 1650, 1400 and 1240 cm^{-1} are caused by the C=O stretching band of carbonyl groups. The phosphate groups show some characteristic adsorption peaks around 1150 and 1078 cm^{-1} representing P=O and P–OH stretching, respectively. The band between 610 and 535 cm^{-1} for the fungal preparation represents C–N–C scissoring and it is only found in protein structure.

Contact angle data with three different test liquids (i.e., water, glycerol and diiodomethane) for the native, heat-, acid- and base-treated fungal biomass and their dye-adsorbed counterpart biomass are tabulated in Table 2. All the tested fungal preparations gave different contact angle values depending on the surface properties. Physical and chemical treatments of the fungal biomass resulted in increase in the hydrophilicity of the fungal biomass compared to native form. The same trend was observed for the dye biosorbed fungal preparations compared to dye-free counterparts. The native form of the fungus was hydrophobic, $\theta > 90$. As seen from the table, after heat, acid or base treatment most of the hydrophobic entities of the fungal cell surfaces were removed as shown by contact angle measurement. It should be noted that physical and chemical treatments change the surface properties with respect to native form. Such changes cause contact angles and later adsorption capacity changes too. The base-treated fungal preparation had lower adsorption capacity for the dye than those of the native, heat- and acid-treated forms (Table 2).

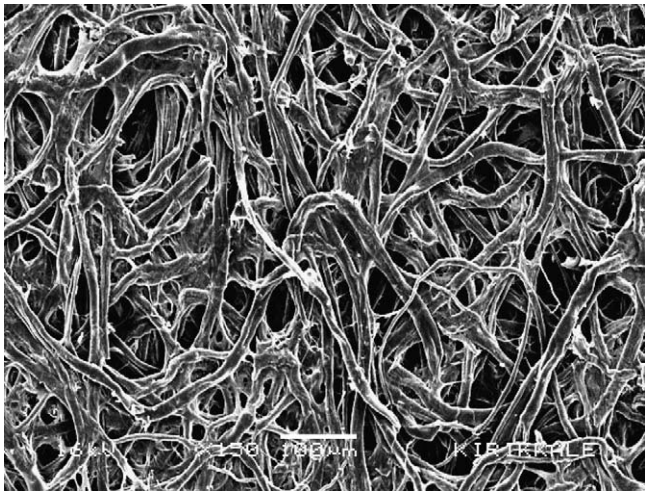

Fig. 2. Representative SEM micrograph of the fungus.

3.2. Effect of pH on biosorption

Textile dyes are complex organic compounds having different aromatic rings and functional groups; the latter have different ionization potentials at different pH and therefore their interaction with microbial biomass depends on the chemistry of a particular dye and the specific chemistry of the biosorbents. The fungal cell wall is composed of polysaccharides (i.e., chitin and chitosan), proteins, lipids and melanin with several functional groups (such as amino, carboxyl, thiol and phosphate groups) capable of binding the dye molecules [28]. The ionic forms of the dye in solution and the surface electrical charge of the biomass depend on the solution pH. Therefore, the interaction between a dye and biosorbent is mainly affected by ionization states of the functional groups on both dye molecule and biosorbent surface [29]. The effect of pH on the dye removal efficiency on the fungal preparations is presented in Fig. 4. The maximum dye biosorption was observed at pH 3.0 for native-, heat- and acid-treated biosorbent. As seen from the figure, as the pH was decreased, the biosorption of Reactive Blue 4 on the fungal biomass preparations increased. Reactive Blue 4 dye molecule has two sulfonate and a primary amino groups (Fig. 7). The pK_a values of the sulfonate and amino groups of the dye molecule are around 0.8 and 7.0, respectively. These functional groups can be easily dissociated and thus, the dye molecule has negative and positive charges in the working experimental conditions. Therefore, the positive and negative sites of the fungal biomass such as protonated form of amino groups (i.e., $–NH_3^+$; pK_a values between 7.0 and 10.0), and deprotonated form of carboxylic and phosphate groups (the pK_a values around 4.0 and 6.5, respectively) can play a role in Reactive Blue 4 biosorption. With increasing pH, the binding sites increase, and thereby the biosorption of Reactive Blue 4 increases. Other researcher has reported similar results. Hu reported that the maximum adsorption of different reactive dyes on the *Aeromonas* sp. biomass was observed at pH 3.0 [30]. O'Mahony et al. studied biosorption of different reactive dyes on *Rhizopus arrhizus* biomass and the maximum dye biosorption was at pH 2.0 [31].

3.3. Effect of physical and chemical treatment on dye biosorption efficiency

Both native and dead biomasses (such as heat inactivated, acid, base and/or otherwise chemically treated) have been used

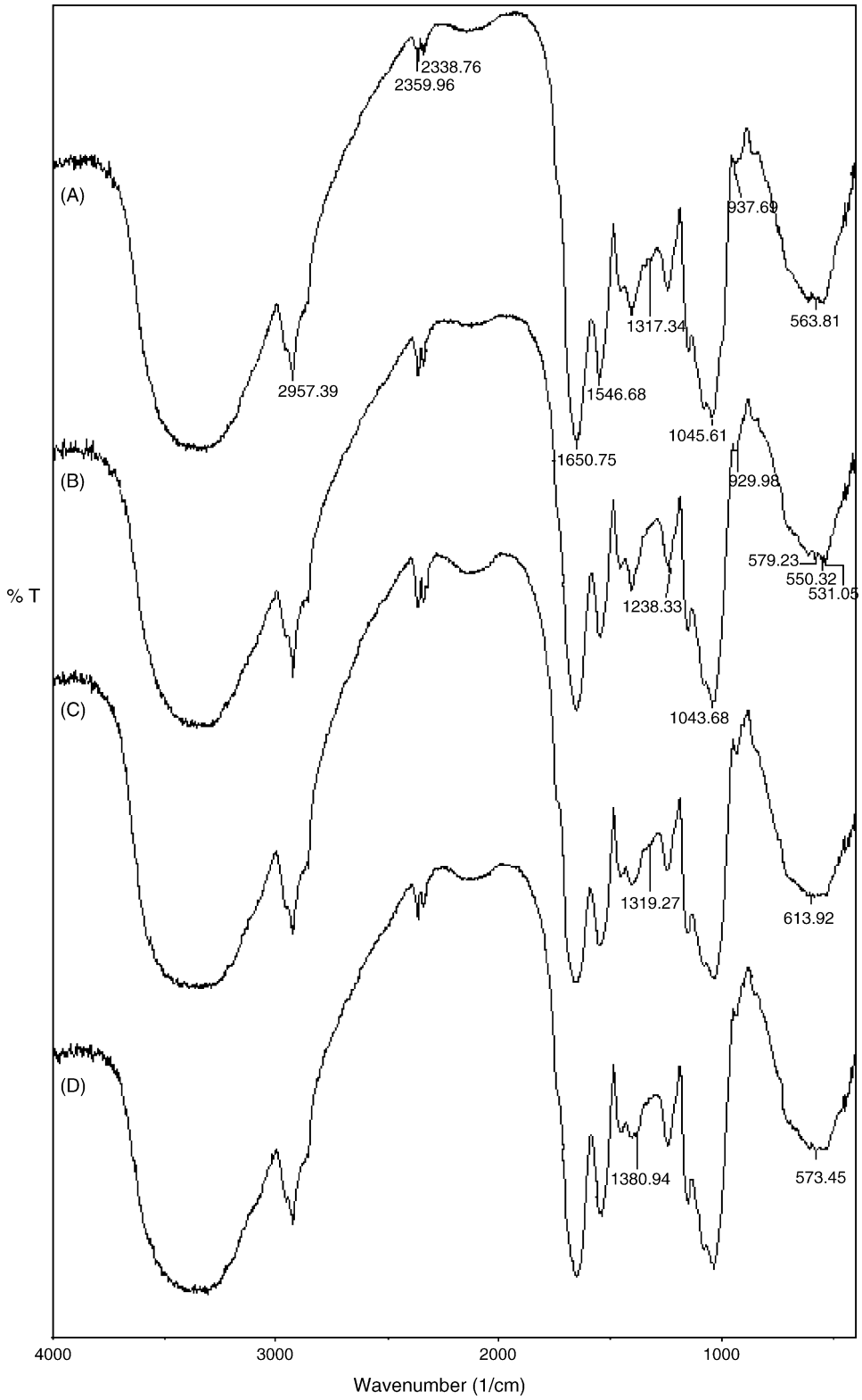

Fig. 3. FTIR spectra: (A) native, (B) acid-treated, (C) base-treated and (D) heat-treated fungal biomass preparations.

to remove hazardous pollutant from aqueous medium [2,6,8]. The use of physically or chemically treated microbial biomass in biosorption is more advantageous for water treatment in that dead microorganisms 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 [32]. The biosorption capacity of the native fungal biomass for Reactive Blue 4 dye was 132.5 mg (g dry biomass)$^{-1}$. The biosorption capacities of the acid- and heat-treated fungal biomass were increased about 1.11- and 1.18-fold compared to native form, respectively, whereas a

Table 2
Contact angles of various test liquids for the tested fungal preparations

Fungal biomass	Water, θ (°) (γ_{erg} = 71.3)	Glycerol, θ (°) (γ_{erg} = 64.0)	Diiodomethane, θ (°) (γ_{erg} = 50.8)
Native	99.5 ± 1.1	96.2 ± 1.3	58.6 ± 1.4
Native-dye	55.3 ± 1.7	84.8 ± 2.2	46.8 ± 0.8
Heat-treated	81.8 ± 1.3	83.5 ± 2.1	34.7 ± 1.8
Heat-treated dye	55.3 ± 1.4	83.9 ± 2.4	47.9 ± 1.9
Acid-treated	76.5 ± 2.1	88.5 ± 1.7	31.7 ± 2.2
Acid-treated dye	52.3 ± 1.1	84.0 ± 1.9	43.9 ± 1.4
Base-treated	77.5 ± 0.9	81.7 ± 1.6	32.4 ± 0.9
Base-treated dye	54.5 ± 0.6	79.1 ± 0.8	44.4 ± 1.1

γ_{erg}: surface tension of test liquid.

decrease in the biosorption capacity of the base-treated biomass was observed about 1.63-fold. The highest biosorption capacity, observed with the heat-treated biomass, could be explained by the increase in the additional binding sites via denaturation of proteins on the cell wall surfaces. The acid treatment was observed to be also effective in increasing the biosorption capacity of the fungal biomass. The acid treatment causes degradation of acid labile cell wall components into oligomers (i.e., polysaccharides and protein). On the other hand, the base-treated biomass had a low biosorption capacity for the Reactive Blue 4 dye with respect to other fungal biomass preparations. The base treatment causes hydrolysis of the phospholipids portion of the cell membrane, thus a decrease in availability of the phosphate groups for binding with the dye molecule due to the disintegration of the fungal cell membranes. The biosorption capacity order of the fungal biomass preparations was observed as follows: heat-treated > acid-treated > native > alkali-treated.

3.4. Influence of temperature on biosorption

The temperature of the biosorption medium could be important for energy dependent mechanisms in dye removal by microbial biomass. The effect of temperature on the equilibrium biosorption capacity of the native and/or treated fungal biomass preparations was studied in the temperature range of 4–37 °C at an initial dye concentration of 200 mg L^{-1}. As shown in Fig. 5, the biosorption of the dye increased with increasing temperature from 4 to 37 °C due to increased surface activity and increased kinetic energy of the dye molecules. Similar observations were reported in the literature [33]. For example, Aksu and Cagatay reported that the adsorption capacity of *Chlorella vulgaris* for Ramazol Black B dye was increased as the temperature increased [5].

The rate constants (k_2) were obtained from Ritchie kinetic equation at different temperatures and were applied to estimate the activation energies of the fungal biomass preparations. The Ritchie constant is expressed as a function of temperature by the following Arrhenius type relationship:

$$\ln k_2 = \ln k_o - \frac{E_a}{RT}$$

where E_a (kJ mol^{-1}) is the activation energy of sorption, k_o (g mg^{-1} min^{-1}) the temperature independent factor, R (8.314 J mol^{-1} K^{-1}) the gas constant and T (K) is the solution temperature. The magnitude of E_a may give idea about the type of adsorption (i.e., physical or chemical) [35]. In physical sorption, the equilibrium is usually rapidly attained and the

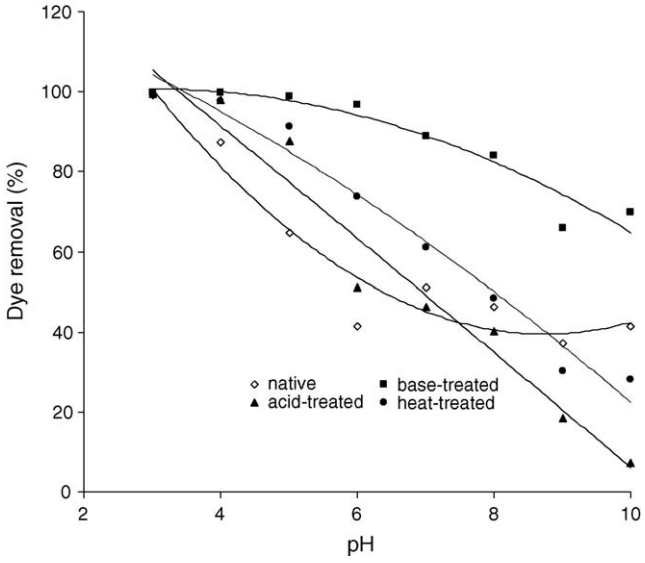

Fig. 4. Effect of pH on the biosorption capacities of the native, heat-, acid- and base-treated fungal biomass preparations for Reactive Blue 4 (initial concentration of dye: 200 mg L^{-1}; temperature: 25 °C).

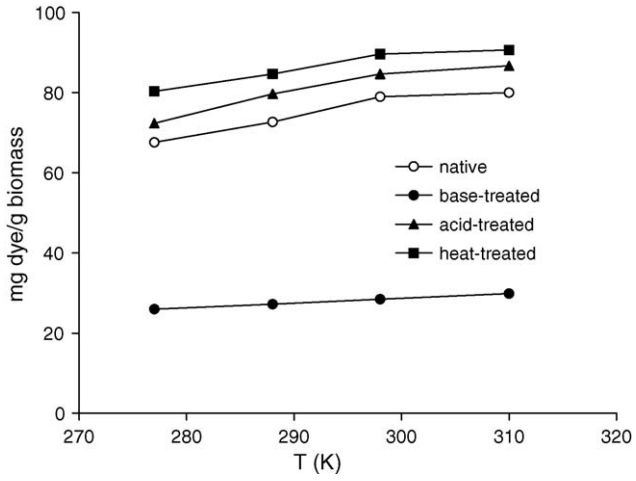

Fig. 5. Biosorption capacities of native, heat-, acid- and base-treated fungal biomass preparations for Reactive Blue 4 at different temperatures (initial concentration of dye: 200 mg L^{-1}; pH: 3.0).

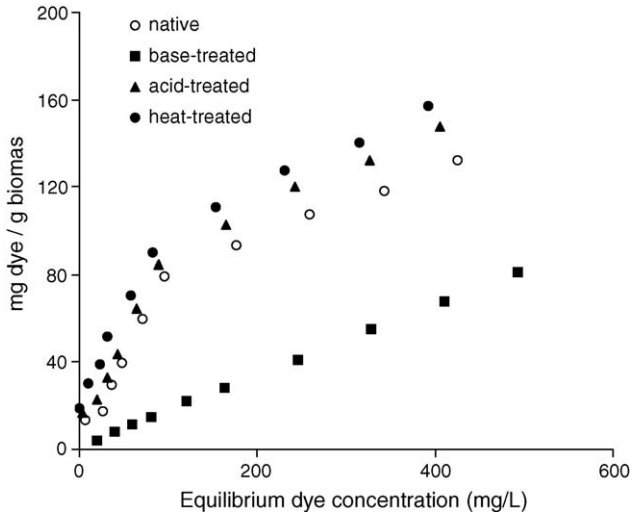

Fig. 6. The biosorption capacities of the native heat-, acid- and base-treated fungal biomass preparations for Reactive Blue 4 at different equilibrium dye concentrations (temperature: 25 °C; pH: 3.0).

activation energy for physical adsorption is usually no more than 4.2 kJ mol^{-1}. On the other hand, chemical sorption is specific and involves force much stronger than in physical sorption. Chemical sorption means that the rate varies with temperature according to finite activation energy in the Arrhenius equation. The activation energies of the native and heat-, acid- and base-treated biomass systems for biosorption of Reactive Blue 4 were found as 7.9, 15.6, 13.7 and 5.0 kJ mol^{-1}, respectively, from the slope of line plotted by ln k_2 against 1/T. These values are of the same magnitude as the activation energy of chemical sorption.

3.5. Biosorption isotherms

Equilibrium data, commonly known as adsorption isotherms, are the basic requirement for the design of adsorption systems. Equilibrium dye concentration provides an important driving force to overcome all mass transfer resistances of the dye between solid and liquid interface, so lower equilibrium concentration of dye molecules may reduce the biosorption. The amount of biosorbed Reactive Blue 4 onto the fungal biomass preparations was studied and plotted as a function of the equilibrium concentration of dye in the biosorption medium (Fig. 6). The biosorption capacities for the dye with native and heat-, acid- and base-treated biomass were determined as 134.5, 156.9, 147.7 and 81.1 mg (g dry fungal biomass)$^{-1}$, respectively, under given experimental conditions (Fig. 6). The most widely used three isotherm models (viz. the Langmuir, Freundlich and Temkin) were used for modeling experimental equilibrium data. The Langmuir isotherm model was not found suitable, but the Freundlich and the Temkin models were able to describe the biosorption equilibrium of the dye on native, physically and chemically treated fungal biomass preparations. Attempts to use the Langmuir equation, non-linear Scatchard plot the biosorption heterogeneity to fit the biosorption isotherm failed to provide a satisfactory correlation. The results show that the Freundlich model was adequately described, indicating no clear adsorption saturation (Table 3). K_F, one of the Freundlich constants, has been used as a relative measure of adsorption capacity and it can be also considered as an indicative parameter of the adsorption strength. As seen from the table, all calculated values of K_F showed easy uptake of the dye on all the tested fungal biomass preparations. The n values are >1 for all the tested fungal biomass preparations, indicating a favorable adsorption process.

The Temkin model predicts a uniform distribution of binding energies over the population of surface binding sites. The range and distribution of binding energies should depend strongly on the density and distribution of functional groups, both on the dye and on the biosorbent surfaces. The model parameters from Temkin isotherm obtained non-linear regression are given in Table 3. The Temkin isotherm model also fitted the experimental adsorption data well, which indicated important role for adsorption played by combined mechanisms onto heterogeneity in the surface.

3.6. Biosorption kinetic models

The experimental kinetic data of biosorption studies were applied to the first-order and Ritchie kinetic models. First-order kinetic indicates that the process of biosorption occurs at a rate proportional to dye concentration, which is particularly suitable for low concentrations. Ritchie kinetic model is thought to drive from biosorption processes in which the rate-controlling step is an exchange reaction [34,35]. The rate constants, k, for the biosorption of the dye on native and/or treated biomass preparations were determined from the first- and second-order rate equation given by Lagergren and Ritchie models and are tabulated in Table 4. The data obtained by the Lagergren first-order kinetic equation were not well-described biosorption of the dye on all the tested biomass preparations. The Ritchie kinetic model was suitable for description of biosorption kinetic for the removal of Reactive Blue 4 from aqueous solution onto native and heat-, acid- and base-treated biomass of *P. chrysosporium*. The experimental values of maximum biosorption capacity (q_{exp}) for the

Table 3
The Freundlich and Temkin models constants and correlation coefficients for biosorption of Reactive Blue 4 on the fungal biomass preparations

Fungal biomass	Freundlich constants			Temkin constants			
	n	K_F	R^2	Q_T (mg g^{-1})	K_T ($\times 10^4$, M^{-1})	R^2	ΔG (kJ mol^{-1})
Native	1.31	1.12	0.988	40.3	2.23	0.977	−24.81
Heat-treated	1.47	2.14	0.996	45.6	2.51	0.978	−25.10
Acid-treated	1.38	1.53	0.993	44.1	2.29	0.974	−24.87
Base-treated	1.06	0.19	0.999	23.8	1.63	0.931	−24.03

Table 4
The first-order and Ritchie kinetic constants for biosorption of Reactive Blue 4 on the fungal preparations

Fungal biomass	Experimental	First-order kinetic			Ritchie kinetic		
	q_{exp} (mg g^{-1})	k_1 ($\times 10^{2-}$, (min^{-1})	q_{eq} (mg g^{-1})	R^2	k_2 ($\times 10^2$, (g mg^{-1} min^{-1})	q_{eq} (mg g^{-1})	R^2
Native	132.5 ± 4.5	2.34	114.8	0.980	5.75	133.3	0.993
Heat-treated	156.9 ± 2.9	1.54	100.6	0.898	8.51	158.3	0.996
Acid-treated	147.7 ± 4.8	1.94	97.1	0.957	6.72	151.8	0.993
Base-treated	81.1 ± 3.2	2.23	69.2	0.980	6.51	79.7	0.991

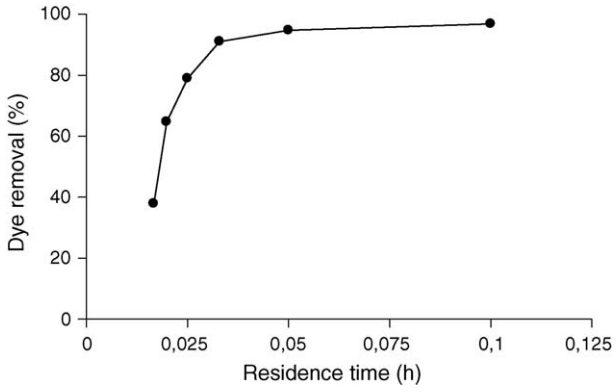

Fig. 7. Effect of residence time on the biosorption efficiency of the dye for heat-treated fungal biomass in the continuous system.

dye on all the tested fungal biomass preparations are very close to calculated theoretical values (q_{eq}) of the Ritchie kinetic model, and also indicated that this process followed the Ritchie kinetic model (Table 4).

The K_i values are calculated for intra-particle model and tabulated in Table 5 for the tested fungal biomass preparations. The linearity of the plots showed that intra-particle diffusion might have a significant role in the biosorption of the Reactive Blue 4 on the biomass preparations. These plots should have zero intercept if the intra-particle diffusion is the sole rate-limiting step. This was not the case in the presented work (the plots had intercepts between 28.08 and 70.83). From this observation, intra-particle diffusion cannot be the dominating mechanism for the biosorption of the dye from aqueous solution by the native and modified biomass of *P. chrysosporium* biomass.

3.7. Continuous system studies

The effect of flow rate on the biosorption of Reactive Blue 4 by the heat-treated *P. chrysosporium* biomass was investigated by fixing initial dye concentration 400 mg L^{-1} and keeping flow rate between 20 and 120 mL h^{-1}. Fig. 7 shows the effect of residence time on the adsorption efficiency of the dye by the heat-treated fungal biomass. The results show that the biosorption of dye molecules on to the fungal biomass was dependent on the residence time. As seen in the figure, the residence time is increased; the removal efficiency is also increased. At the highest flow rate, the lowest dye removal efficiency was observed. This behavior can be due to the insufficient contact time between the dye and the fungal biomass.

Table 5
The intra-particle diffusion rate constants for biosorption of Reactive Blue 4 on the fungal preparations

Fungal biomass	K_i	R^2
Native	43.05	0.898
Heat-treated	28.08	0.912
Acid-treated	60.86	0.875
Base-treated	70.83	0.880

Variation of the biosorption rate with varying the dye concentration in the continuous system led to a linear increase in the biosorption efficiency which was observed up to 400 mg L^{-1} dye concentration and after a plateau between 400 and 600 mg L^{-1} was obtained (data not presented). The residence time was calculated as 0.02 h for the continuous system at a flow rate of 100 mL h^{-1} and, this was 4.0 h for the batch system. In both systems, the same series initial dye concentration was used. In the continuous system, the adsorption capacity of the heat-treated fungal biomass was determined as 211.6 mg g^{-1}. On the other hand, the biosorption capacity for the same preparation was obtained as 156.9 mg g^{-1} from the batch system. In the continuous system, the adsorption capacity of the fungal biomass was about 35% higher than that of the batch system. The observed lower biosorption capacity in the batch systems may be a result of the low equilibrium concentration of the dye compared to continuous system. In the continuous system, the concentration of the dye in the effluent increased gradually until the adsorptive sides of the biomass saturated with the dye molecules. At saturation, the concentration of dye in the effluent was same as the inlet dye concentration. The driving force for biosorption is the concentration difference between the dye on the biosorbent and the dye in the solution [4,36]. Thus, the high driving force due to high dye concentration resulted in a better performance in the continuous system.

4. Conclusions

The results of this study clearly showed that physical and chemical surface modification methods can be used to maximize the dye removal efficiency of the fungal biomass. Biosorption is strongly pH dependent and decreases markedly above pH 3.0. The biosorption of the dye on the fungal biomass preparations increased with increasing temperatures under given experimental conditions. The biosorption capacities of the native, heat-, acid- and base-treated fungal biomass preparations for the dye were 132.5, 156.9, 147.6 and 81.1 mg (g dry biomass)$^{-1}$. The present biosorption values are comparable with dye binding by other fungal biomass preparations. The biosorption capac-

ity of *R. arrhizus* biomass was between 130 and 180 mg (g dry biomass)$^{-1}$ [4]. Tobin and co-workers reported that the maximum biosorption capacities of *R. arrhizus* biomass for Remazol Blue, Remazol Orange and Cibacron red were 90, 190 and 150 mg (g dry biomass)$^{-1}$, respectively [31]. The biosorption capacities of dead macro fungus *Phellinus igniarius* for biosorption of Methylene Blue and Rhodamine were 232.7 and 36.8 mg (g dry biomass)$^{-1}$, respectively [29]. The Freundlich and Temkin isotherm models were more applicable to the type of biosorption achieved by fungal biomass preparations. The interaction of the dye with the fungal biomass preparations could be better explained by the second-order kinetic model. A series of continuous system studies revealed that flow rate and initial dye concentration affected dye biosorption on the fungal biomass. These results suggest that heat- and acid-treated *P. chrysosporium* biomasses have potential applications in biotreatment systems for the dye removal from the effluent of the textile industry. Further studies may be needed for the removal of other hazardous materials present in textile effluents such as heavy metal ions and other textile dyes.

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