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Biosorption of benzidine based textile dyes "Direct Blue 1 and Direct Red 128" using native and heat-treated biomass of *Trametes versicolor*

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Abstract

The capacities and mechanisms of native and heat-treated white rot fungus "*Trametes versicolor*" biomass in removing of two different benzidine based dyes (i.e., Direct Blue 1, DB-1 and Direct Red 128, DR-128) from aqueous solution was investigated with different parameters, such as molecular weight of dye, adsorbent dosage, pH, temperature and ionic strength. In the batch system, the biosorption equilibrium time for both dyes was about 6 h. The maximum biosorption was observed at pH 6.0 for DB-1 and at pH 3.0 for DR-128 on the native and heat-treated fungal biomass. The biosorption capacities of the native and heat-treated fungal biomass (at 800 mg/L dye concentration) were found to be 101.1 and 152.3 mg/g for DB-1 and these were 189.7 and 225.4 mg dye/g biomass for DR-128, respectively. The Freundlih and Temkin adsorption isotherm models were used for the mathematical description of the biosorption equilibrium. The Freundlich and Temkin models were able to describe the biosorption equilibrium of DB-l and DR-128 on the native and heat-treated fungal preparations. The Freundlich model also showed that the small molecular weight dye (i.e., DR-128) had a higher affinity of adsorption that than of the higher molecular weight dye (i.e., DB-1). The dye biosorption on the fungal biomass preparations followed the second order kinetics model.

Keywords: *Trametes versicolor*; Biosorption; Heat-treatment; Benzidine based dye; Kinetics; Adsorption isotherm models

1. Introduction

Pollutants are described as the substances or energy that causes hazards to human health, harm to living resources and ecological systems or damage to structure or amenity. Concerns of their interference with the legitimate uses of the environment have never been alleviated over years due both to the high amount and variety of such substances in use. Dyes, for example, being not an exemption among many others, are represented by more than 10,000 chemically different types and the estimated production of them is 7×10^8 kg/year [1–4]. Dye related effluents from a wide variety of industries such as textile, tannery, packed food, pulp and paper, paint and electroplating, threaten the life in much aspects. Direct dyes are water-soluble molecules containing one or more ionic groups (most often sulfonic acid and/or amino groups). Among them, benzidine based direct dyes or their metabolites (e.g., aromatic amines) in dyes can be highly toxic and potentially carcinogenic since the various modifications to the molecule in production of different dyes do not seem to significantly reduce that potential [5,6]. As such, they are expected to metabolize to benzidine, which is a known human carcinogen. In the absence of specific data benzidene-based dyes are all classed as "known to be carcinogens". The presence of a benzidine element in a molecule is thus to be regarded as a serious alert of potential carcinogenicity [7].

Color that inherently occurs in water bodies receiving dye contaminated effluents can significantly affect 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 [5,8–10]. Having usually synthetic origin and complex aromatic molecular structures, dye components are hardly degradable [11,12]. The majority of technologies based on physicochemical processes such as dilution, adsorption, coagulation and flocculation, chemical

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precipitation, oxidation, ion-exchange, reverse osmosis and ultra filtration [13] are presently employed for color removal from the aquatic media. However, high cost, formation of hazardous by-products, intensive energy requirements and inefficient reusability of adsorbents are still limitations commonly countered during the application of these techniques [14–17]. Among treatment technologies, adsorption has been shown to be the most promising option for the removal of non-biodegradable organics from aqueous effluents, activated carbons being the most common adsorbent for this process due to its effectiveness and versatility [18]. But, activated carbon has also some disadvantages. Both chemical and thermal regeneration of used carbon is expensive, impractical on a large scale and produces additional effluent and results in considerable loss of the adsorbent. Attempts made to search for the use of cheap and efficient alternative materials such as bagasse pith [19], peat [20], fly ash [15], saw dust [21], etc., have produced results with low adsorption capacity and disposal problem of large quantity of sorbents.

Since the 1980s, the adaption of live or dead biomass or their derivatives into adsorption studies has successfully been made, especially for the removal of heavy metals and other pollutants from wastewater [22–25]. These biosorption studies exhibit comparable performance at a very low cost to other available counterparts such as precipitation, ion exchange, reverse osmosis and adsorption. The high efficiency in the reusability of biological adsorbent is of significance for the compatibility of biosorption technology [22].

Biomass of natural microbial species, including bacteria, fungi, and algae is capable of removing the different textile dyes by biosorption, biodegradation, or mineralization [26]. The uptake or accumulation of chemicals by microbial mass is termed biosorption and may involve a combination of active and passive transport mechanism starting with the diffusion of the adsorbed component to the surface of the microbial cell. Binding to sites at the cell surface which exhibit chemical affinity for the adsorbate is generally non-metabolism dependent and so occurs for both viable and inactivated microbial cells [3,18]. Some low cost fungal materials have been used as biosorbent for dye and metal ions from aqueous water or wastewater, which included *Lentinus sajor caju* [26], *Trametes versicolor* [27], *Lentinus edodes* [28], *Aspergillus niger* [30]. The white rot fungus, *T. versicolor* has been widely applied for bioremediation studies [27,31]. However, a few studies have been focused on utilization of the white rot fungus for dye removal.

In this study, the native and heat-treated biomasses of *T. versicolor* were used for the biosorption of two different direct dyes (i.e., Direct Blue 1 and Direct Red 128) in batch system. The purpose of the heat-treatment is to improve the surface characteristics of the fungal biomass in relation to their dye adsorbing mechanisms. It was then assumed that the pretreatment may lead to an increase of the charge on the cell surface or open the available sites for the adsorption and enhance ion-exchange. The biosorption kinetics was further studied to explore the effects of initial dye concentration, temperature and pH on dye removal. The biosorption phenomena were expressed by the Freundlich and Temkin adsorption models. The experimental data was also analyzed using the first- and second order kinetic models.

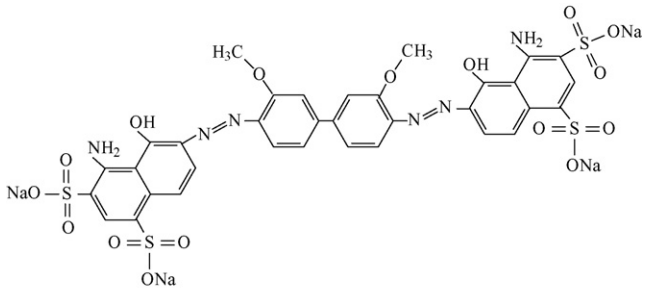

Fig. 1. The chemical structure of the Direct Blue 1 and Direct Red 128.

2. Materials and methods

2.1. Materials

Direct Blue 1 (Pontamine Sky Blue; 3-amino-5-[(4-{4-[(8-amino-1-hydroxy-5,7-disulfo(2-naphthyl))diazenyl]-3-methoxyphenyl}-2-methoxyphenyl)diazenyl]-4-hydroxy naphthalen-1,2,5,8-tetra-sulfonic acid) and Direct Red 128 (Congo Red; 4-Amino-3-[(4-{4-[(1-amino-4-sulfo(2-naphthyl))diazenyl]phenyl}phenyl)diazenyl]naphthalenesulfonic acid) were obtained from Sigma–Aldrich Chemical Co, St. Louis, MO, USA. The chemical structure and some properties of the DB-1 and DR-128 dyes 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 and preparation of biomass T. versicolor

The white rot fungus *T. versicolor* was cultivated in liquid medium using the shake flask method. The growth medium consisted of (g/L of purified water); D-glucose (10.0); KH_2PO_4

Table 1
The general characteristics of Direct Blue 1 and Direct Red 128

Name of dyes	Pontamine Sky Blue	Congo Red
Color index name	CI. Direct Blue 1	CI. Direct Red 128
Color index number	24410	22120
Chemical formula	$C_{34}H_{28}N_6O_{16}S_4{\cdot}4Na$	$C_{32}H_{22}N_6O_6S_2{\cdot}2Na$
H-bond donors	8	4
H-bond acceptors	22	12
Molar mass	992.80	696.7
λ_{max} (nm)	505	498

(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. Heat treated form of *T. versicolor* was prepared in physiological saline solution by heating at 100 °C for 10 min and after treatment referred as heat-treated fungal biomass. Native and heat 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.

2.3. Biosorption studies

The biosorption of DB-1 and DR-128 on the native and heat-treated fungal biomass preparations were investigated in a batch system. A stock solution (1000 mg/L) of each dye was obtained by dissolving the dyes in purified water. The ranges of concentrations of both dyes were prepared from these stock solutions. To determine the effect of initial concentrations of the dyes on the biosorption rate and capacity on the fungal preparations, the initial concentration of each dye was changed between 25 and 800 mg/L in the biosorption medium.

The effects of the initial medium pH on the biosorption capacity of the fungal biomass preparations was investigated in the pH range of 2.0–9.0 (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., 5, 15, 25 and 35 °C) and at pH 6.0. In a typical biosorption experiment, fungal biomass (250 mg wet weight) in 50 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. Before analysis of the remaining dye concentration, the fungal biomass was collected by centrifugation at 3000 rpm and was dried under reduced pressure in a vacuum oven at 60 °C for 24 h. The amount of biosorbed dye per unit fungal biomass (mg dye per g dry biomass) was obtained by using the following expression:

$$q = \frac{(C_0 - C)V}{M} \tag{1}$$

where q is the amount of dye biosorbed onto the unit amount of the biomass (mg/g); C_0 and C are the concentrations of the each dye in the initial solution (mg/L) and after biosorption, respectively, V the volume of the aqueous phase (L) and M is the amount of the biomass (g). The concentrations of each dye in the samples were analyzed using a double beam UV/vis spectrophotometer (Shimadzu, Tokyo, Japan; Model 1601) at 505 and 498 nm for DB-1 and DR-128, respectively. Results given in averages were obtained from the experiments repeated three times.

2.4. Adsorption isotherm models

The Freundlich adsorption isotherm model has been shown to describe the biosorption equilibrium. The equations can be written in the form given below to predict the adsorption capacities of the biosorbent:

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

K_F and n are the Freundlich adsorption isotherm constants characteristic of the system. K_F and n are indicative of the extent of the adsorption and the degree of non-linearity between solution concentration and adsorption, respectively. On the other hand, the Temkin isotherm model assumes that adsorption is characterized by a uniform distribution of binding energies, up to some maximum binding energy (ΔG_{max}), which results in the following isotherm equation [32]:

$$Q = Q_T \ln(1 + K_T C) \tag{3}$$

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

2.5. Determination of kinetic parameters

The kinetics of dye biosorption on the fungal biomass preparations was determined with two different kinetic models, i.e., the first- and second order. The first-order rate equation of Lagergren is one of the most widely used equations for the sorption of solute from a liquid solution [33]. It may be represented as follows:

$$\frac{dq_t}{dt} = k_1(q_{eq} - q_t) \tag{4}$$

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

$$\log\left(\frac{q_{eq}}{q_{eq} - q_t}\right) = \frac{k_1 t}{2.30} \tag{5}$$

A plot of $\log(q_{eq} - q_t)$ against t should give a straight line to confirm the applicability of the kinetic model. In a true first order process $\log q_{eq}$ should be equal to the intercept of a plot of $\log(q_{eq} - q_t)$ against t.

Ritchie proposed a method for the kinetic adsorption of gases on solids [34]. If the dye biosorption medium is considered to be a second-order reaction, Ritchie equation is:

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

where k_2 (g/(mg min)) is the rate constant of the second order adsorption.

A plot of $1/q_t$ versus $1/t$ (Eq. (6)) should give a linear relationship for the applicability of the second-order kinetic. The rate constant (k_2) and adsorption at equilibrium (q_{eq}) can be obtained from the intercept and slope, respectively, and there is no need to know any parameter beforehand.

2.6. Characterization of the fungal biomass

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

(ii) *The FT-IR spectra*: The IR spectra of native and heat-treated *T. versicolor* were obtained by using a FT-IR spectrophotometer (Mattson 1000 FT-IR, England). For FT-IR spectra, approximately 0.01 g biosorbent sample was encapsulated in 0.1 g KBr and pressed into a tablet form by pressing the ground mixed material with the aid of a bench press. The FT-IR spectrum was then recorded.

(iii) *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 fungal 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 groups (i.e., amino, carboxyl and phosphate) on the fungal biomass, the FT-IR spectra of the native and heat-treated fungal biomass were obtained. The spectra for fungal biomass preparations are presented in Fig. 3. In general, the FT-IR spectra of both 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.

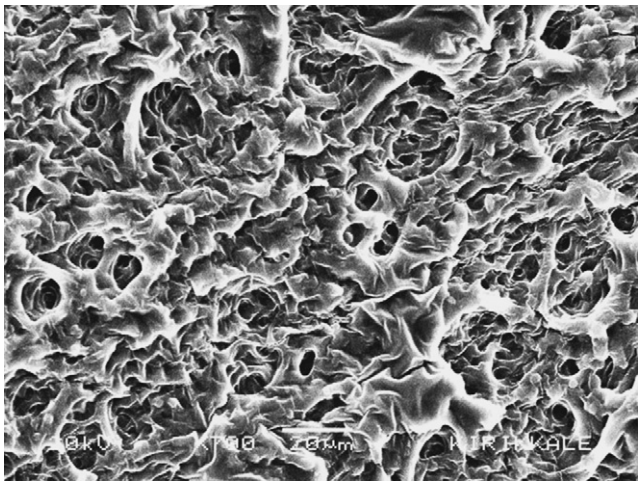

Fig. 2. Representative SEM micrograph of the fungus.

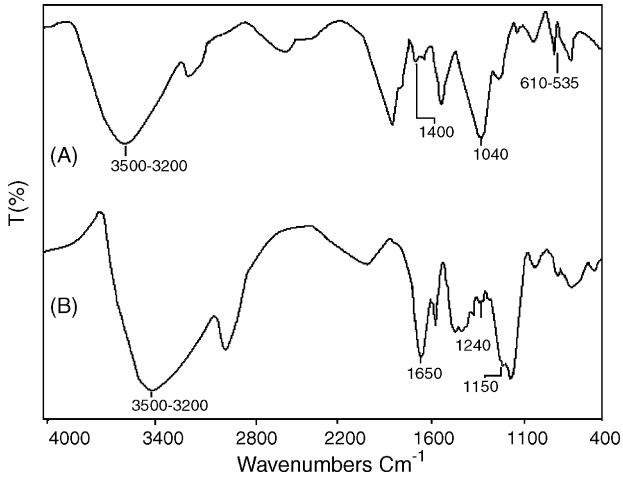

Fig. 3. FT-IR spectra of the (A) native and (B) heat-treated fungal biomass.

Some changes can be observed comparing the native fungal biomass with that after heat-treated counterpart. A significant shift can be seen in contract with the fungal biomass before and after heat-treatment (Fig. 3). For example, the peaks around 1650, 1400 and 1150 cm^{-1} strengthened, shifted and/or broadened, indicating that heat-treatment process was effective

Table 2
Contact angles of various test liquids for the tested *Trametes versicolor* preparations

Fungal biomass	Water θ (°) (γ_{erg} = 71.3)	Glycerol θ (°) (γ_{erg} = 64.0)	Diiodomethane θ (°) (γ_{erg} = 50.8)
Native	103.6 ± 1.4	98.2 ± 0.8	59.3 ± 1.1
Native-DB-1	67.8 ± 1.5	88.4 ± 2.3	43.5 ± 0.5
Native-DR-128	61.7 ± 1.7	82.2 ± 2.3	49.4 ± 1.2
Heat-treated	82.2 ± 0.8	85.3 ± 1.2	33.1 ± 1.1
Heat-treated-DB-1	64.5 ± 2.1	81.6 ± 0.8	39.5 ± 0.7
Heat-treated-DR-128	61.4 ± 0.9	77.8 ± 2.1	39.5 ± 0.8

γ_{erg}: surface tension of test liquid.

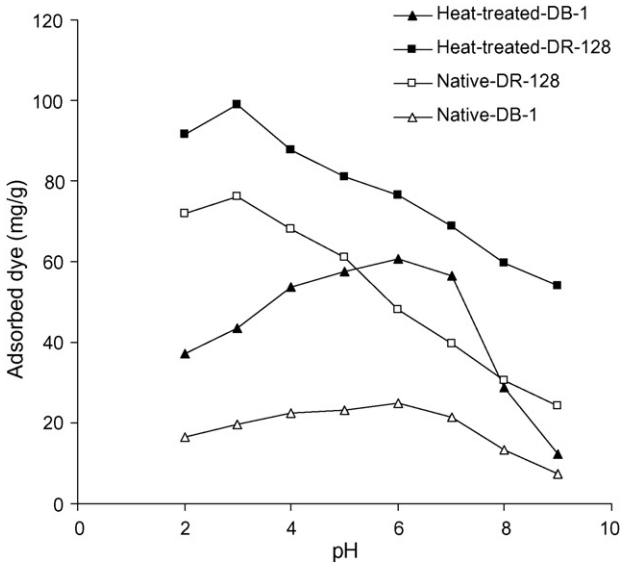

Fig. 4. Effect of pH on the biosorption capacities of the native and heat-treated fungal biomass for Direct Blue 1 and Direct Red 128 (initial concentration of each dye: 200 mg/L; temperature: 25 °C).

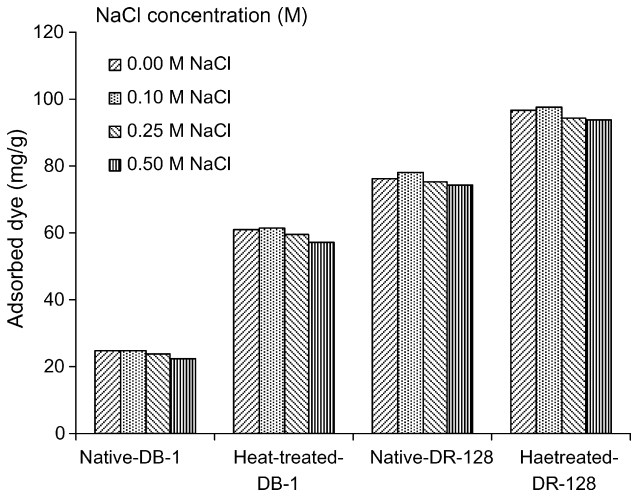

Fig. 5. Effect of ionic strength on the biosorption capacities of the native and heat-treated fungal biomass for Direct Blue 1 and Direct Red 128 (initial concentration of each dye: 200 mg/L; pH 3.0 for DR-128 and 6.0 for DB-1; temperature: 25 °C).

for the modification of the surface properties of the fungal biomass.

Contact angle data with three different test liquids (i.e., water, glycerol and diiodomethane) for the native and heat-treated fungal biomass and their dye-adsorbed counterpart are tabulated in Table 2. The tested fungal samples gave different contact angle values depending on the surface properties. Heat-treatment resulted in increase in the hydrophilicity of the fungal biomass surface 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 $\theta > 90$. After heat-treatment, most of the hydrophobic entities on the fungal cell surfaces were removed as shown by contact angle measurement. It should be noted that heat-treatment change the surface properties compared to native form. Such changes cause contact angles and later adsorption capacity changes too.

3.2. Effect of pH on biosorption

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 [5,30]. 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 [35,36]. The effect of pH on the benzidine based dyes removal efficiency on the native and heat-treated fungal biomass is presented in Fig. 4. As seen in the figure, the pH profile and biosorption capacity of the fungal biomass preparations for each dye was different from each other. The biosorption of DB-1 increased with pH up to 6.0 and then declined with further increase in pH and, the maximum equilibrium uptake value was found to be 24.8 mg/g for native and 60.8 mg/g for heat inactivated fungal biomass with an initial dye concentration of 200 mg/L. On the other hand, the equilibrium binding capacity of DR-128 dye also increased to the value of 76.3 mg/g for native and 98.9 mg/g for inactivated fungal biomass by increasing pH value from 2.0 to 3.0. The enhancement of uptake of direct dyes at acidic pH values may be explained in terms of electrostatic interactions between the fungal biomass preparations and the dye molecules. The present results are comparable with dye binding by other biosorbent materials. For example, Fu and Viraraghavan [1] reported that initial pH of dye solution significantly influenced the chemistry of both Acid Blue 29 and Basic Blue 9 dyes molecules and the inactivated fungal biomass of *A. niger*. The effective initial pH was obtained at 6.0 and 4.0, for Acid Blue 29 and Basic Blue 9 dyes, respectively. Aksu and Tezer reported that the maximum Remazol Black B biosortion was obtained at pH 2.0 on the dried fungal biomass of *Rhizopus arrhizus*. They explained the higher uptakes at lower pH values by electrostatic attractions between negatively charged dye anions and positively charged cell surface [37].

3.3. Effect of ionic strength

The enthalpy of biosorption would be affected not only by the pH value on the electron donating capability, but also by the salt concentration on the hydrophobic and electrostatic interaction between dye and surface functional adsorptive sites of the fungal biomass preparations. The adsorption capacities of the native and heat-treated fungal biomass to both benzidine-based dyes were not significantly affected with increasing NaCl concentration from 0 to 0.5 M (Fig. 5). It should be noted that an increased ionic strength with NaCl did not interfere in the studied experimental range with the binding of both dyes to the fungal biomass preparations. Dyeing processes consume large amounts of salt. Therefore, the concentration of salt in dye wastewaters can be normally high. From this point of view, this result indicates that the biomass of *T. versicolor* can be used

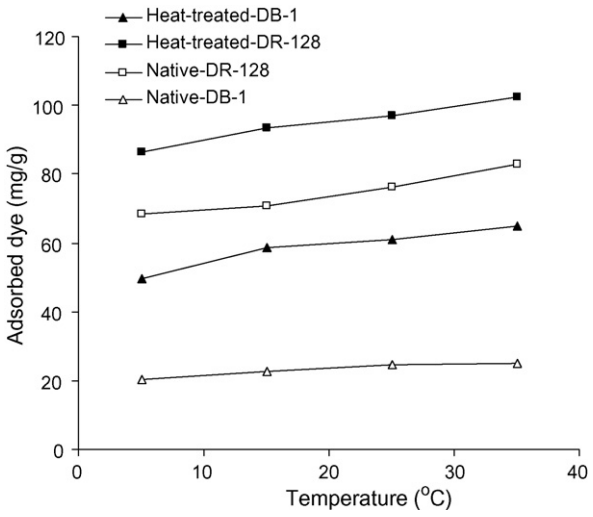

Fig. 6. Biosorption capacities of native and heat-treated fungal biomass at different temperatures for Direct Blue 1 and Direct Red 128 (initial concentration of each dye: 200 mg/L; pH 3.0 for DR-128 and 6.0 for DB-1).

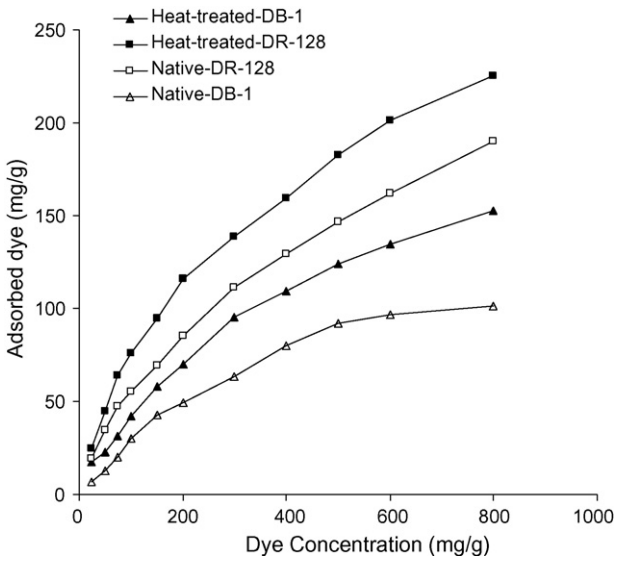

Fig. 7. Effect of initial dye concentration on the biosorption capacities of the native and heat-treated fungal biomass for Direct Blue 1 and Direct Red 128 (initial concentration of each dye: 200 mg/L; pH 3.0 for DR-128 and 6.0 for DB-1; temperature: 25 °C).

for removal of benzidine based direct dyes from salt containing water.

3.4. Effect of temperature

Various textile dye effluents are produced at relatively high temperature, therefore, temperature can be an important factor for the real application of the fungal biomass. The effect of temperature on the equilibrium biosorption capacity of the native and heat-treated fungal biomass was studied in the temperature range of 5–35 °C at an initial dye concentration of 200 mg/L. As shown in Fig. 6, the biosorption capacities of the fungal preparations were increased for both dyes with increasing temperature from 5 to 35 °C due to increased surface activity and increased kinetic energy of each dye molecules [18,38]. Since the biosorption increased when temperature increased, therefore, the system was endothermic. Similar observations were reported in the literature [38,39]. For example, Aksu and Tezer reported that the adsorption capacity of the green alga *Chlorella vulgaris* for Ramazol Black B was increased with increases in temperature [37].

3.5. Effect of initial dye concentration on biosorption

The amount of biosorbed DB-1 and DR-128 onto the native and heat-treated fungal biomass preparations at equilibrium were studied and plotted as a function of the initial concentration of each dye in the biosorption medium (Fig. 7). The biosorption capacity of the biomass preparations increased with increasing of the initial concentration of both dye in the biosorption medium. The maximum biosorption capacities of the native and heat-treated fungal biomass were found to be 101.1 and 152.3 mg/g (or 0.102 and 0.153 mmol/g) for the DB-1 and 189.7 and 225.4 mg/g (or 0.271 and 0.322 mmol/g) for DR-128 dye dry fungal biomass, respectively (Fig. 7). The heat-treated form demonstrated higher biosorption capacity to both dyes than those of the native form. These results may be explained by the increase in the additional binding sites via denaturation of proteins on the cell wall structures. In the biosorption studies of two different dyes by native and heat treated fungal biomass, the amino groups could be the major binding site, while carboxylic acid and phosphate groups as well as lipid fraction might also provide additional binding sites [4]. As seen from Fig. 1, both dyes molecules have sulphonate and amino groups and can be ionized easily in aqueous solution. Therefore, the positively and negatively charged functional groups on the fungal biomass can interact with opposite charges of dyes molecules under given experimental condition. It should be noted that, molecular weight of dye molecules has an important influence on the removal efficiency and biosorption capacity of native and/or treated biomass. As seen in Fig. 7, the low biosorption of DB-1 in comparison to that of DR-128 may be attributed molecular mass of the dyes and restricted its adsorption. As given in Table 1, the molecular weight of DB-1 is much higher than that of the DR-128 dye, which will prevent the molecules entering the smaller pores of the biosorbents, resulting in lower adsorption capacity. The present results also compare favorable with the dye binding to other biosorbents, which are used in the dye removal studies from aqueous solutions. For example, O'Mahony et al. [3] investigated the effect of dye concentration on the adsorption of Remazol Blue, Remazol Orange and Cibacron Red using *R. arrhizus* biomass and, the uptake capacity of the microbial biomass was 90, 190 and 150 mg per dye g biomass, respectively.

3.6. Dyes biosorption isotherms

The Freundlich and Temkin isotherm models were used to fit the experimental data and these isotherm models are usually adopted for heterogeneous adsorption. The experimental,

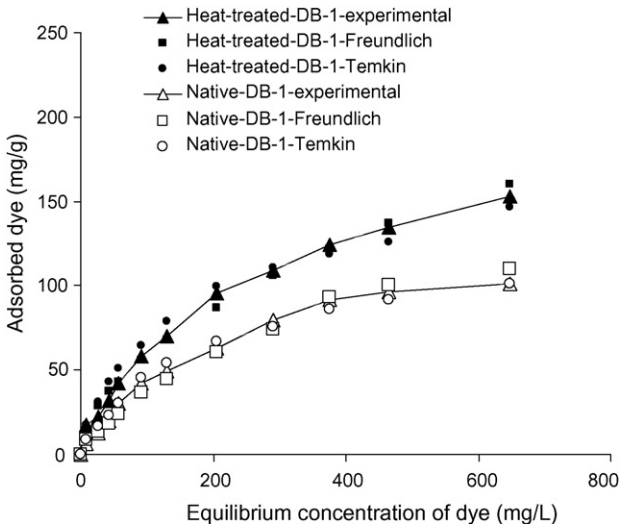

Fig. 8. Comparison of the equilibrium experimental and the adsorption isotherms obtained from the Freundlich and Temkin models for DB-1 adsorption on the native and heat-treated fungal biomass.

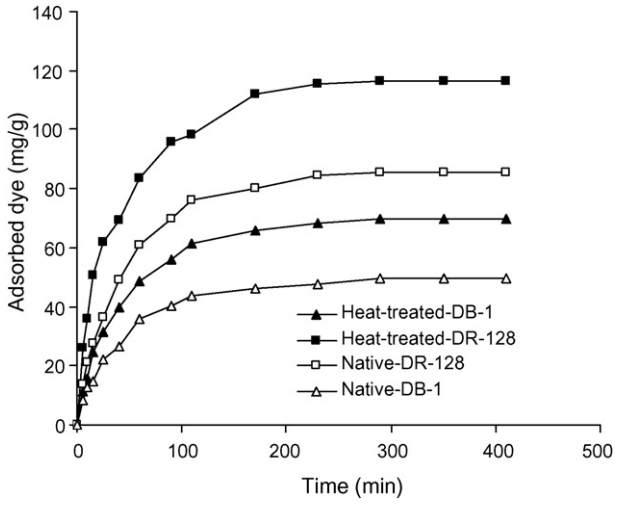

Fig. 9. The equilibrium adsorption time of DR-128 and DB-1 biosorption onto native and heat-treated fungal biomass (initial concentration of each dye: 200 mg/L; pH 3.0 for DR-128 and 6.0 for DB-1; temperature: 25 °C).

Freundlich and Temkin isotherms are exemplified for DB-1 dye and, is presented in Fig. 8. The experimental isotherm was fitted by the Freundlich and Temkin isotherm models. The Freundlich isotherm relates the adsorbed concentration as the power function of solute concentration. In this isotherm model, the magnitude of K_F and n values of the model showed easy uptake of dyes from aqueous medium with a high adsorption capacity of the fungal biomass preparations. Values of $n > 1$ for both dyes molecules indicates positive cooperativity in binding and a heterogeneous nature of adsorption (Table 3). In the case of the Temkin model, the corresponding semi-logarithmic plots gave rise to linear plot for the biosorption of both dyes, to fungal biomass preparations and the correlation coefficient of the semi-logarithmic plots (R^2) was above 0.988 for the biomass preparations, indicating the Temkin model best fitted the experimental data (Table 3). For the interactions between each dye molecules and fungal biomass surface should result in uniform binding energies, up to some maximum binding energy (ΔG_{max}). In these cases, ΔG_{max} values were found to be between −27.19 and −26.10 kJ/mol for the DB-1 and DR-128 dyes on the fungal biomass preparations (Table 3). From these results, the range and distribution of binding energies should depend on the density, and type of functional groups, both on the dye molecules and the fungal biomass preparations surface. As indicated in the Temkin model, the binding energy decreased with increasing the amounts of adsorbed dye on the biosorbents surface (Table 3).

3.7. Biosorption equilibrium time and kinetic models

Biosorption rates of DB-1 and DR-128 were obtained by recording the gradual decrease of the concentration of each dye within the adsorption medium with time (Fig. 9). The time necessary to reach equilibrium for the removal of the dye molecules by native and heat-treated treated fungal biomass from aqueous solution was established about 6 h. After equilibrium, the amount of adsorbed dye did not change significantly with time.

The experimental kinetic data of biosorption studies were applied to the first and second order 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. Second order kinetic is thought to drive from biosorption processes in which the rate-controlling step is an exchange reaction [17]. The rate constants, k, for the biosorption of the dye molecules on native and heat-treated biomass preparations were determined from the first- and second order rate equation given by Lagergren and Ritchie and tabulated in Table 4. The data obtained by the Lagergren first order kinetic equation did not describe well the biosorption of the tested dye on the biomass preparations. On the other hand, the experimental value of maximum biosorption capacity (q_{exp}) for the DB-1 and DR-128 dyes on the fungal preparations are very close to calculated theoretical values (q_{eq}) of the second order kinetic model, and also indicated that this process followed the second-order kinetic model (Fig. 10 and Table 4). These results suggest that

Table 3
The Freundlich and Temkin models constants and correlation coefficients for biosorption of DB-1 and DR-128 on the native and heat-treated fungal biomass

Fungal biomass	Freundlich constants			Temkin constants			
	n	K_F	R^2	Q_T (mg/g)	$K_T \times 10^4$ (M^{-1})	R^2	ΔG (kJ/mol)
Native-DB-1	1.18	1.51	0.983	28.9	4.11	0.988	−26.10
Heat-treated-DB-1	1.24	1.78	0.972	43.2	3.42	0.995	−27.03
Native-DR-128	1.41	1.84	0.967	66.5	3.14	0.992	−27.69
Heat-treated-DR-128	1.32	1.54	0.967	54.0	3.22	0.993	−27.19

Table 4
The first-order and second-order kinetics constants for biosorption of DB-1 and DR-128 on the native and heat treated fungal biomass

Fungal biomass	Experimental	First-order kinetic			Second-order kinetic		
	q_{exp} (mg/g)	$k_1 \times 10^2$ (min^{-1})	q_{eq} (mg/g)	R^2	$k_2 \times 10^2$ (g/(mg min))	q_{eq} (mg/g)	R^2
Native-DB-1	101.1	2.17	120.1	0.993	2.98	99.6	0.997
Heat-treated-DB-1	152.3	3.09	171.8	0.978	3.51	153.3	0.971
Native-DR-128	189.7	1.83	160.9	0.998	4.56	186.5	0.989
Heat-treated-DR-128	225.4	2.19	212.1	0.993	5.21	222.3	0.991

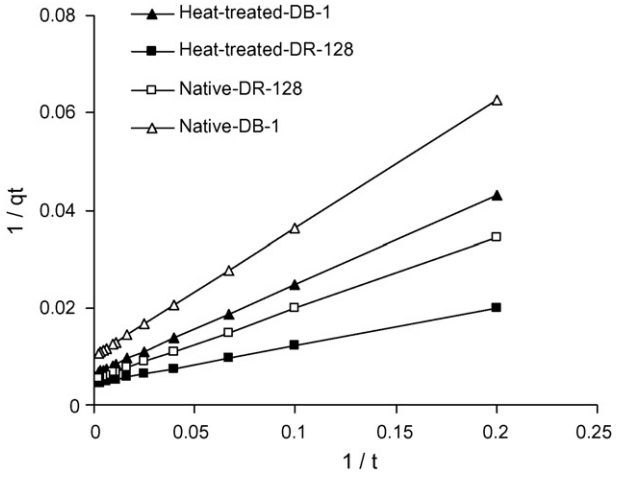

Fig. 10. The second-order kinetic plots for adsorption of DR-128 and DB-1 onto native and heat-treated fungal biomass.

the second order mechanism is predominant and that chemisorption might be the rate-limiting step that controls the biosorption process. The rate-controlling mechanism may vary during the course of the biosorption process three possible mechanisms may be occurring. There is an external surface mass transfer or film diffusion process that controls the early stages of the adsorption process. This may be followed by a reaction or constant rate stage and finally by a diffusion stage where the adsorption process slows down considerably [40,41].

Thus, the second order kinetic model was suitable for description of biosorption kinetics for the removal of DB-1 and DR-128 dyes from aqueous solution onto native and heat-treated biomass of *T. versicolor*.

4. Conclusions

The biosorption of Direct Blue 1 and Direct Red 128 dyes from aqueous solution on the native and heat-treated fungal biomass preparations was studied in a batch system with respect to medium pH, temperature and initial dye concentration. The results of the study clearly showed that physical surface modification methods could be used to maximize the dye removal efficiency of the fungal biomass. Biosorption of each dye is strongly pH dependent. The maximum biosorption capacities of the native and heat-treated fungal biomass were found to be 101.1 and 152.3 mg/g for the DB-1 and 189.7 and 225.4 mg/g for DR-128 dye dry fungal biomass, respectively. The Temkin isotherm model was more applicable to the type of biosorption achieved by native and heat-treated fungal biomass of *T. versicolor*. The first and second order kinetic models were applied to the biosorption of the dye on the fungal biomass preparations and it was observed that the interactions could be better explained on the basis of second order kinetic equations. These results suggest that heat-treated *T. versicolor* biomasses have potential applications in biotreatment systems for the dye removal from the effluent of the textile industry. It may be concluded that fungal biomass can be operated in the continuous flow stirred tank or column reactor configurations for large-scale application and, the fungal biomass can be recovered from these systems by simple filtration. Such applications would involve easily the replacement of conventional adsorbent materials by biosorbent. A comparison of biosorption capacity of *T. versicolor* with those obtained in the literature for the adsorption of textile dyes on alternative biosorbents also showed that *T. versicolor* is more effective for this purpose [10,12,16,29]. 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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References

[1] Y. Fu, T. Viraragvahan, Dye biosorption sites in *Aspergillus niger*, Biores. Technol. 82 (2002) 139–145.
[2] Y.C. Toh, J.J.L. Yen, P.O. Jeffrey, Y.P. Ting, Decolourization of azo dyes by white-rot fungi (WFR) isolated in Singapore, Enzyme Microb. Technol. 35 (2003) 569–575.
[3] T. O'Mahony, E. Guibal, J.M. Tobin, Reactive dye biosorption by *Rhizopus arrhizus* biomass, Enzyme Microb. Technol. 31 (2002) 456–463.
[4] K.V. Radha, A. Regupathi, T. Arunagiri, T. Murugesan, Decolarization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics, Process Biochem. 40 (2005) 3343–3377.
[5] G. Bayramoğlu, G. Çelik, M.Y. Arica, Biosorption of Reactive Blue 4 dye by native and treated fungus *Phanerocheate chrysosporium*: batch and continuous flow system studies, J. Hazard. Mater. 137 (2006) 1597–1689.
[6] L.K. Lowry, W.P. Tolos, M.F. Boeniger, C.R. Nony, M.C. Bowman, Chemical monitoring of urine from workers potentially exposed to benzidine-derived azo dyes, Toxicol. Lett. 71 (1980) 29–36.
[7] E. Rinde, W. Troll, Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey, J. Natl. Cancer Inst. 55 (1975) 181–182.

[8] A. Ozer, G. Akkaya, M. Turabik, The removal of acid red 274 from wastewater: Combined biosorption and bio-coagulation with *Spirogyra rhizopus*, Dyes Pigm. 71 (2006) 83–89.
[9] X.Y. You, J.G. Chen, Y.N. Hu, Studies on the relation between bladder cancer and benzidine or its derived dyes in Shanghai, Brit. J. Ind. Med. 47 (1990) 544–552.
[10] T. Akar, T.A. Demir, I. Kiran, A. Ozcan, A.S. Ozcan, S. Tunali, Biosorption potential of Neurospora crassa cells for decolorization of acid red 57 (AR57) dye, J. Chem. Technol. Biotechnol. 81 (2006) 1100–1106.
[11] Y. Fu, T. Viraraghavan, Dye biosorption sites in *Aspergillus niger*, Biores. Technol. 82 (2002) 239–247.
[12] K.V. Radha, A. Regupathi, T. Arunagiri, T. Murugesan, Decolarization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics, Process Biochem. 40 (2005) 3345–3377.
[13] I.M. Banat, P. Igam, D. Singh, R. Marchant, Microbial decolorization of textile-dye-containing effluents: a review, Biores. Technol. 58 (1996) 217–227.
[14] M.Y. Arica, G. Bayramoglu, Cr(IV) biosorption from aqueous solution using free and immobilized biomass of *Lentinus sajor-caju*: peraparation and kinetic characterization, Colloid Surf. A: Physicochem. Eng. Aspects 253 (2005) 203–211.
[15] V.K. Gupta, D. Mohan, S. Sharma, M. Sharma, Removal of basic dyes (Rhodamine B and Methylen Blue) from aqueous solutions using bagasse fly ash, Sep. Sci. Technol. 35 (2000) 2097–2113.
[16] S.R. Couto, M.A. Sanroman, D. Hofer, G.M. Gubitz, Stainless steel sponge: a novel carrier for immobilization of the white rot fungus Trametes hirsute for decolorization of textile dyes, Biores. Technol. 95 (2004) 67–72.
[17] M.Y. Arıca, G. Bayramoglu, M. Yılmaz, O. Genc, S. Bektas, Biosorption of Hg^{+2}, Cd^{+2} and Zn^{+2} by Ca-alginate and immobilized wood-rotting funguns *Funalia trogii*, J. Hazard. Mater. 109 (1–3) (2004) 191–199.
[18] Z. Aksu, Application of biosorption for the removal of organic pollutants: a review, Process Biochem. 40 (2005) 997–1026.
[19] M.M. Nassar, M.S. El-Geundi, Comparative cost of color removal from textile effluents using natural adsorbents, J. Chem. Technol. Biotechnol. 50 (1991) 257–264.
[20] K.R. Ramakrishna, T. Viraghavan, Dye removal using peat, Am. Dyestuff Report. 85 (1996) 28–30.
[21] T.H. El-Nabarwy, S.A. Khedr, Removal of pollutants from water using untreated and treated sawdust and water hyacinth, Adsorp. Sci. Technol. 18 (2000) 385–398.
[22] O. Yesilada, D. Asma, S. Cing, Decolorization of textile dyes by fungal pellets, Process Biochem. 38 (2003) 933–938.
[23] G. Bayramoglu, G. Celik, M.Y. Arica, Studies on accumulation of uranium by fungus *Lentinus sajor-caju*, J. Hazard. Mater. 136 (2006) 343–351.
[24] G. Bayramoğlu, I. Tuzun, G. Celik, M. Yilmaz, M.Y. Arıca, Biosorption of Hg(II), Cd(II) and Pb(II) ions by microalgae *Chlamydomonas reinhardtii* immobilised in alginate beads, Int. J. Min. Process 81 (2006) 35–43.
[25] M.Y. Arica, I. Tuzun, E. Yalçın, O. Ince, G. Bayramoglu, Utilisation of native, heat and acid-treated microalge *Chlamydomonas reinhardtii* preparations for biosorption of Cr(VI) ions, Process Biochem. 40 (2005) 2351–2358.
[26] G. Bayramoglu, G. Celik, E. Yalcın, M. Yilmaz, M.Y. Arica, Modification of surface properties of *Lentinus sajor-caju* mycelia by physical and chemical methods: evaluation of their Cr^{+6} removal efficiency from aqueous medium, J. Hazard. Mater. 119 (2005) 219–229.
[27] G. Bayramoglu, S. Bektas, M.Y. Arica, Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*, J. Hazard. Mater. 101 (2003) 285–300.
[28] G.-Q. Chen, G.-M. Zeng, X. Tu, C.-G. Niu, G.-H. Huang, W. Jiang, Application of a by product of *Lentinus edodes* to the bioremediation of chromate contaminated water, J. Hazard. Mater. 135 (2006) 249–255.
[29] A. Ozer, G. Akkaya, M. Turabik, Biosorption of Acid Red 274 (AR 274) on *Enteromorpha prolifera* in a batch system, J. Hazard. Mater. 126 (2005) 119–127.
[30] Y. Fu, T. Viraraghavan, Removal of Congo Red from an aqueous solution by fungus *Aspergillus niger*, Adv. Environ. Res. 7 (2002) 239–247.
[31] P. Baldrian, Interactions of heavy metals with white-rot fungi, Enzyme Microb. Technol. 32 (2003) 78–91.
[32] G. Bayramoglu, B. Kaya, M.Y. Arica, Procion Brown MX-5BR attached and Lewis metal ion immobilized poly(hydroxyethylmethacrylate)/chitosan IPNs membranes: their lysozyme adsorption equilibria and kinetic characterization, Chem. Eng. Sci. 57 (2002) 2323–2334.
[33] S. Lagergren, About the theory of so-called adsorption of soluble substance, Kung Sven. Veten. Hand. 24 (1898) 1–12.
[34] A.G. Ritchie, Alternative to the Elovich equation for kinetics of adsorption of gases on solids, J. Chem. Soc. Faraday Trans. 73 (1977) 1650–1657.
[35] T.V.N. Padmesh, K. Vijayaraghavan, G. Sekaran, M. Velan, Biosorption of Acid Blue 15 using fresh water macroalga *Azolla filiculoides*: batch and column studies, Dyes Pigm. 71 (2006) 77–82.
[36] N.S. Maurya, A.K. Mittal, P. Cornel, E. Rother, Biosorption of dyes using dead macro fungi: effect of dye structure, ionic strength and pH, Biores. Technol. 97 (2006) 512–521.
[37] Z. Aksu, S. Tezer, Biosorption of reactive dyes on the green alga *Chlorella vulgaris*, Process Biochem. 40 (2005) 1347–1361.
[38] A. Aretxaga, S. Romero, M. Sarra, T. Vicent, Adsorption step in the biological degradation of a textile dye, Biotechnol. Progr. 17 (2001) 664–671.
[39] Z. Aksu, S.S. Cagatay, Investigation of biosorption of Gemazol Turquise Blue-G reactive dye by dried *Rhizopus arrhizus* in batch and continuous systems, Sep. Purif. Technol. 48 (2006) 24–35.
[40] T. Akar, S. Tunali, Biosorption characteristics of *Aspergillus flavus* biomass for removal of Pb(II) and Cu(II) ions from an aqueous solutions, Biores. Tecnol. 97 (2006) 1780–1787.
[41] C.-Y. Chen, C.-L. Chiang, P.-C. Huang, Adsorption of heavy metal ions by a magnetic chelating resin containing hydroxyl and iminodiacetate groups, Sep. Purif. Technol. 50 (2006) 15–21.