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A Comparative Study on Biosorption Characteristics of Certain Fungi for Bromophenol Blue Dye

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Abstract

Laboratory investigations of the potential use of dried biomasses of *Rhizopus stolonifer*, *Fusarium* sp., *Geotrichum* sp., and *Aspergillus fumigatus* as biosorbents for the removal of bromophenol blue (BPB) dye from aqueous solutions were conducted. Kinetics studies indicated that the BPB dye uptake processes can be well described by the pseudo-second-order model. The fungal biomasses exhibited the highest dye biosorption at pH 2.0. The Langmuir adsorption model appears to fit the dye biosorption better than the Freundlich model, with maximum dye uptake capacities ranging from 526 to 1111 mg/g, depending on the biomass used.

Index Entries: Biosorption; bromophenol blue; fungi; isotherms; kinetics.

Introduction

Large amounts of dyes are produced annually and used in the textile, cosmetic, paper, leather, pharmaceutical, food, and other industries *(1)*. However, dyeing industry effluents constitute one of the most problematic wastewaters to be treated, not only because of their high chemical and biologic oxygen demands and content in toxic compounds, but also

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because of their color, which is the first contaminant to be recognized by the human eye *(2–4)*.

Dye wastewater is usually treated through physical or chemical treatment processes. These include chemical coagulation/flocculation, oxidation, precipitation, and adsorption. Some of these techniques have been determined to be effective, although they have limitations. Among these are an excess amount of chemical usage, or accumulation of concentrated sludge with obvious disposal problems; expensive plant requirements, or operational costs; lack of effective color reduction; and sensitivity to a variable wastewater input *(5–7)*. Therefore, innovative treatment technologies are currently being investigated.

Biosorption is one such process on which an increased amount of study is being focused *(8)*. Biosorption of dyes consists of an adsorptive binding of dyes to the inactive dead biomass, using purely physicochemical pathways of uptake *(9)*. The biologic materials that have been investigated for dye removal include fungi *(10)*, bacteria *(11)*, yeast *(12)*, and other biomasses *(13–15)*. The implied mechanisms in biosorption differ quantitatively and qualitatively according to the type of biomass, its origin, and its processing.

The biosorption of dyes using various fungal biomasses has been investigated *(4,16–18).* These studies indicated that fungal biomass has a positive potential for the development of cost-effective biosorbents. Fungi are easily grown and produce high yields of biomass. Furthermore, fungi are extensively used in a variety of large-scale industrial fermentation processes, and waste biomass from these processes is a potential source of cheap biosorbent material *(19)*. Compared with live fungal cells, dead fungal biomass provides various advantages, such as absence of nutrient needs, ease of regeneration, and reuse for many cycles of treatment *(20)*.

In the present study, biosorption of bromophenol blue (BPB) dye (3',3",5',5"-tetrabromophenolsulfonphtalein, sodium salt) by four dried fungi biomasses was investigated. This dye, which is a triphenylmethane derivative, and its structurally related compounds, such as fluoresceins and xanthenes, are widely used as industrial dyes for foods, drugs, cosmetics, textiles, printing inks, or laboratory indicators. Some of these compounds have been reported to be genotoxic *(21,22)*.

Materials and Methods

Microorganisms and Growth Conditions

Rhizopus stolonifer, Fusarium sp., *Geotrichum* sp., and *Aspergillus fumigatus* were isolated from dye-contaminated wastewater. These four fungal strains were identified based on the visual observation of isolates grown on potato dextrose agar (PDA) plates, micromorphologic studies in slide culture *(23)* at room temperature, and the taxonomic keys described in Hoog and Guarro *(24)* as well as the compendium of soil fungi *(25)*.

Stock cultures of isolated strains were routinely maintained on a Luria-Bertani (LB) agar supplemented with 1% glucose. Precultures of each fungus were prepared by inoculating plugs (0.5-cm diameter) from the growing zone of fungus on an agar plate to 50 mL of LB liquid medium supplemented with 1% glucose. Then, cells were cultivated statically at 25°C for 3 d. Afterward, the precultures were homogenized aseptically using a Potter homogenizer (200 rpm), and the homogenates were used as an inoculum for liquid cultures. Liquid cultures consisted of 150 mL of LB supplemented with 1% glucose in 250-mL Erlenmeyer flasks. These were inoculated at 1% with homogenized precultures and incubated aerobically at 25°C on a rotary shaker at 150 rpm for 4 d.

Preparation of Biomasses

The biomass of each fungal strain was separated from the culture broth by filtration, washed three times with distilled water, and then pretreated with NaOH solution (0.1 *M*) for 1 h at room temperature (25°C). The pretreated biomasses were washed with generous amounts of distilled water and autoclaved for 20 min at 121°C and 18 psi. Thereafter, the biomasses were dried overnight at 50°C. The dry biomasses were ground using a mortar, and the powder particles including those between 300 and 500 μm were used as biosorbent.

Preparation of Dye Solution and Dye Analysis

The dye used was BPB (molecular weight = 691.9, λ_{max} = 433 and 590 nm), an anionic triphenylmethane dye, supplied by Sigma-Aldrich (St. Louis, MO). Dye solutions were prepared by dissolving accurately weighted dye in distilled water. To compare dye removal on the same basis, the pH of all the samples was adjusted to 2.0 before measurement with 1 m*M* HCl solution. The concentrations of BPB dye in the biosorption medium were measured spectrophotometrically at 433 nm. Dilution of the dye solution was conducted where required.

Batch Biosorption Studies

Dye uptake studies were conducted in 250-mL Erlenmeyer flasks containing 100 mL of dye solutions with 1 g of biomass/L. The flasks were agitated on a shaker at 150 rpm and left to equilibrate for 20 h at 25°C. The solution pH before and during the sorption tests was adjusted with 1 m*M* NaOH and 1 m*M* HCl solutions. Aliquots of dye solutions were collected at predetermined time intervals to determine the residual dye concentration in the solution. Before analysis the samples were centrifuged at 5000 rpm for 5 min, and the supernatant liquid was analyzed for any remaining dye. All the experiments were conducted in duplicates. Dye adsorbed by the biomass was calculated according to a material balance.

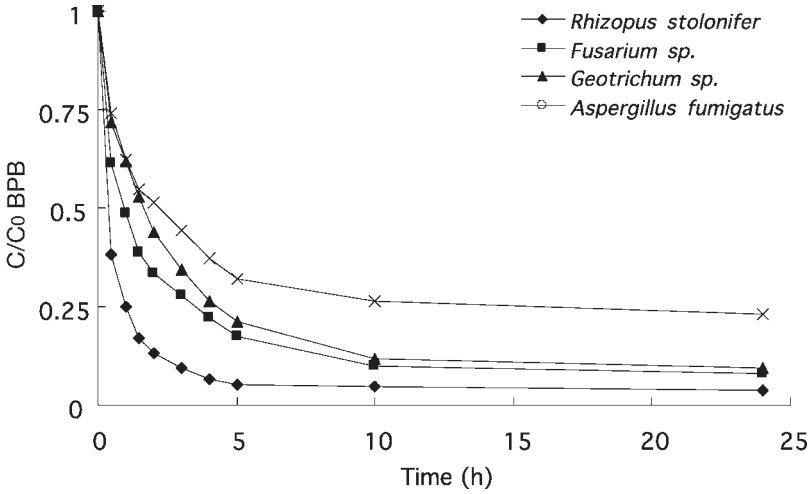

Fig. 1. Uptake kinetics of BPB dye of isolated fungal strains.

Results and Discussion

The kinetics profiles of BPB dye biosorption by *R. stolonifer*, *Fusarium* sp., *Geotrichum* sp., and *A. fumigatus* biomasses were investigated. Biosorption studies were carried out for 24 h in order to determine the effect of contact time on the biosorption of BPB dye at an initial dye concentration of 400 mg/L. From the results, presented in Fig. 1, it can be observed that the BPB dye biosorption kinetics by all the tested biomasses were relatively fast. The kinetics profiles followed the typical fast then slow process in the adsorption system. The fast initial dye sorption rate can be attributed to the surface binding, and the subsequent slower sorption rate can be attributed to interior penetration *(12,26)*. Initial sorption of BPB dye by *R. stolonifer* biomass occurred more rapidly than by the others. A greater amount of dye was removed by these dried cells in the first hour of contact (75%), whereas only 51, 38, and 37% of dye was removed by *Fusarium* sp., *Geotrichum* sp., and *A. fumigatus* biomasses, respectively, for the same contact time. However, more than 90% of BPB dye uptake occurred within 5 h for all tested biomasses. Additionally, equilibrium was established in 15 h for these biosorbents.

The pseudo-first-order Largergren *(27)* and the pseudo-second-order *(28)* were examined to describe the biosorption kinetics of BPB dye on each tested fungal biomass.

The first-order rate expression of Largergren is expressed as follows:

$$\log(q_{eq} - q) = \log_{q_{eq}} - k_{1,ad}t/2.303 \quad (1)$$

in which q is the amount of adsorbed dye per unit weight of adsorbent at time t, q_{eq} is the amount of adsorbed dye per unit weight of adsorbent at equilibrium, and $k_{1,ad}$ is the rate constant of Largergren first-order biosorption.

The pseudo-second-order kinetic rate equation is expressed as follows:

$$t/q = 1/(k_{2,ad}\, q_{eq}^{2}) + t/q_{eq} \quad (2)$$

in which $k_{2,ad}$ is the rate constant of second-order biosorption.

The values of the first-order rate constant ($k_{1,ad}$) and the theoretical equilibrium dye sorption capacity ($q_{eq,cal}$), as well as the correlation coefficients obtained from the pseudo-first-order Largergren model at different initial concentrations of dye (200, 400, and 600 mg/L), were determined and are presented in Table 1. It can be seen from these results that the pseudo-first-order Largergren model, as reflected by the low correlation coefficients obtained and the disparity between the theoretical and experimental equilibrium dye sorption capacities, was not suitable for describing the biosorption kinetics of BPB dye on the studied biomasses. However, the values of the theoretical equilibrium dye sorption capacity ($q_{eq,cal}$) and correlation coefficients obtained from the pseudo-second-order model at the tested initial dye concentrations, presented in Table 1, suggested that the process of dye adsorption followed the second-order rate kinetics. Indeed, using this model, the correlation coefficients were close to the unity for all the concentrations used and the $q_{eq,cal}$ values agreed well with the experimental equilibrium dye sorption capacity. It was also observed that the values of $k_{2,ad}$ decreased with increasing dye concentration with relative rapid biosorption of *R. stolonifer*.

The adsorption of dyes onto the fungal biomass was primarily influenced by the surface charge on the biosorbent, which, in turn, was influenced by the pH of the solution *(29)*. Indeed, the fungal biomass consists of biopolymers with many functional groups, such as carboxyl, amino, and phosphate, of which the electrical charge is pH dependent. To establish the effect of pH on the adsorption of BPB dye onto the four isolated fungal strains, batch equilibrium studies at different pH values were conducted at an initial BPB dye concentration of 800 mg/L. As can be seen in Fig. 2, the biosorptive capacities of all the biomasses presented a similar variation according to pH. The maximum adsorption of the dye was observed at pH 2.0. The biosorption of BPB dye decreased as the pH solution increased from 2.0 to 5.0, and no significant biosorption took place at pH 6.0. This behavior may be explained by the fact that at a pH less than 3.0, the overall surface charge of the fungal cells was positive owing to the protonation of the functional groups. The positively charged sites on the biosorbents favored the approach of negatively charged BPB dye ions, a result of the electrostatic attraction forces. Hydrogen ions also act as a bridging ligand between the fungi cell wall and the dye molecule *(4,30)*. By increasing the pH, more functional groups are dissociated. Consequently, the electronegativity of the biosorbents increases, generating an increase in the repulsion between the dye anions and the negatively charged fungal biomasses, which causes a decrease in biosorption capacities.

Many studies have shown that dye concentration also affects the biosorptive capacity of fungal biomasses as well as the efficiency of color

Table 1
Comparison of First- and Second-Order Rate Constants
and Calculated and Experimental q_{eq} Values Obtained at Different Initial BPB Dye Concentrations for Each Tested Fungal Strain

			First-order model parameters			Second-order model parameters		
	C_0 (mg/L)	$q_{eq,\,exp}$ (mg/g)	$k_{1,ad}$ (L/min)	$q_{eq\ cal}$ (mg/g)	r^2	$k_{2,ad} \times 10^2$ (mg/[g·min])	$q_{eq\ cal}$ (mg/g)	r^2
R. stolonifer	200	191	0.007	90	0.95	0.024	192	0.99
	400	385	0.007	125	0.81	0.019	384	0.99
	600	568	0.006	134	0.74	0.017	588	0.99
Fusarium sp.	200	187	0.008	94	0.95	0.024	192	0.99
	400	368	0.006	246	0.97	0.005	384	0.99
	600	468	0.004	309	0.95	0.003	500	0.99
Geotrichum sp.	200	191	0.004	76	0.96	0.015	196	0.99
	400	363	0.005	277	0.99	0.003	384	0.99
	600	456	0.003	268	0.85	0.002	476	0.99
A. fumigatus	200	180	0.004	104	0.86	0.01	185	0.99
	400	307	0.005	207	0.96	0.004	322	0.99
	600	402	0.004	272	0.92	0.003	434	0.99

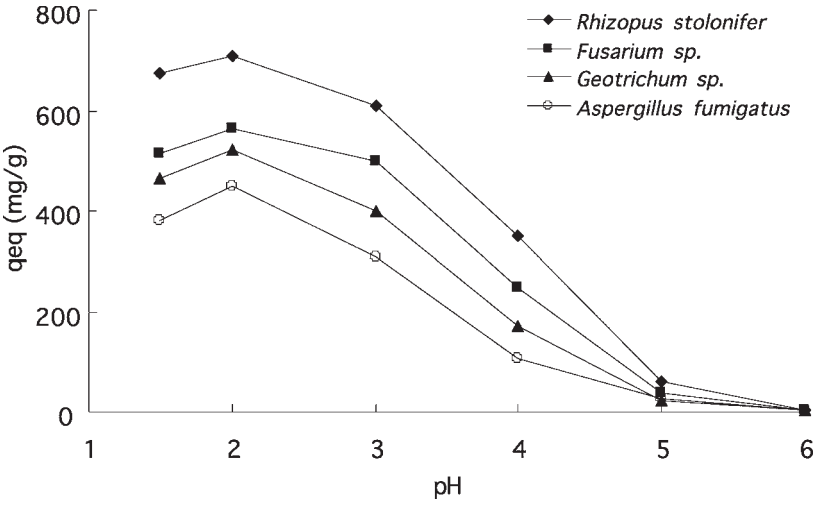

Fig. 2. Effect of pH on equilibrium dye sorption capacity of isolated fungal strains.

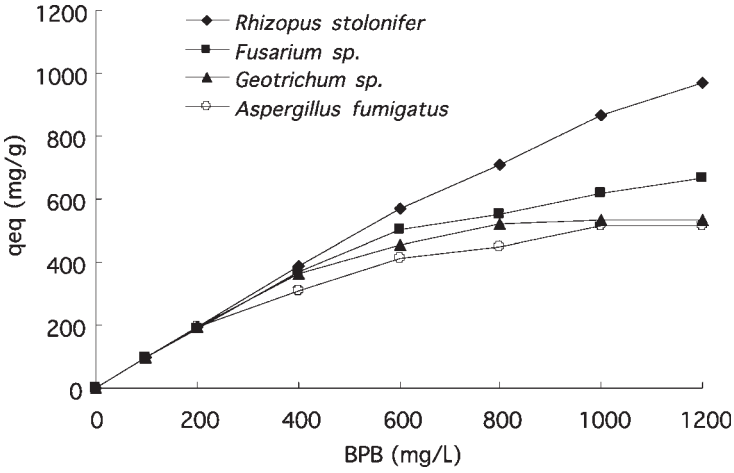

Fig. 3. Effect of initial dye concentration on equilibrium dye sorption capacity of isolated fungal strains.

removal. BPB dye biosorption experiments with each fungal biomass were conducted using solutions at different initial concentrations of dye varying from 100 to 1200 mg/L. As shown in Fig. 3, the uptake capacity of the tested biomasses increased significantly at higher concentrations of dye and the color removal efficiency decreased by increasing the interval dye concentration. Different uptake capacities and removal percentages of BPB dye were observed, depending on the fungal strain used. The highest uptake capacity was registered by *R. stolonifer*, and saturation was not observed in the range of concentrations studied. The uptake capacity of this biosorbent was found to reach 967 mg/g at an initial dye concentration of 1200 mg/L.

The biomass of *R. stolonifer* was capable of removing more than 88% of coloring material up to an initial dye concentration of 800 mg/L. By increasing the initial dye concentration from 200 to 1000 mg/g, the equilibrium sorption capacity increased from 191 to 864 mg/g. For *Fusarium* sp. biomass, when the initial dye concentration increased from 200 to 1000 mg/g, the uptake capacity of the biomass increased from 188 to 619 mg/g and the adsorption yield decreased from 94 to 61%. The maximum BPB dye uptake capacity for *Fusarium* sp. biomass was found to be 666 mg/g at an initial BPB dye concentration of 1200 mg/g. *Geotrichum* sp. and *A. fumigatus* biomasses exhibited the lowest uptake capacity (536 and 518 mg/g, respectively, at 1200 mg/L). By increasing the initial dye concentration from 200 to 1000 mg/L, the adsorption yield decreased from 90 to 47% using *A. fumigatus* biomass and from 95 to 53% using *Geotrichum* sp. biomass. This reduction in the color removal efficiency was owing to the saturation of the sorption sites on the biosorbents as the concentration of the dye increased. Differences in the uptake capacities may be related to compositional differences among the fungal biomasses.

To describe the adsorption isotherm relationships, the experimental equilibrium data of *R. stolonifer*, *Fusarium* sp., *Geotrichum* sp., and *A. fumigatus* were fitted to Langmuir *(31)* and Freundlich *(32)* models.

The Langmuir model is described by the following equation:

$$q_{eq} = q_{\max} b C_{eq} / (1 + b C_{eq}) \tag{3}$$

in which q_{eq} is the quantity of adsorbed dye/g of biomass at equilibrium (mg/g), C_{eq} is the concentration of the dye solution at equilibrium (mg/L), $q_{\max}$ is the maximum amount of dye per unit weight of biomass to form a complete monolayer on the surface bound at C_{eq} (mg/g), and b is a constant related to affinity of the binding sites (L/mg).

The Freundlich model equation is of the following form:

$$q_{eq} = k \cdot C_{eq}^{1/n} \tag{4}$$

in which k and n are the Freundlich constants characteristic of the system.

The maximum uptake capacity $q_{\max}$ and the equilibrium constants b in the Langmuir model as well as k and n in the Freundlich model were regressed from experimental data and are given in Table 2. Values of correlation coefficients showed that the Langmuir model fits better the experimental data than the Freundlich model for all the tested biomasses. The suitability of the Langmuir model shows that the biosorption process is monolayer and has constant adsorption energy. *R. stolonifer* biomass showed the highest maximal uptake capacity for BPB dye biosorption (1111 mg/g) according to the Langmuir model. It was followed by *Fusarium* sp., *Geotrichum* sp., and *A. fumigatus*, with maximum uptake capacities of 714, 588, and 526 mg/g, respectively.

Table 2
Regressed Langmuir and Freundlich Sorption Model Parameters

	Langmuir parameters			Freundlich parameters		
	q_{max} (mg/g)	b (L/mg)	r^2	k	n	r^2
R. stolonifer	1111	0.026	0.99	80.26	2.16	0.88
Fusarium sp.	714	0.028	0.99	75.52	2.76	0.87
Geotrichum sp.	588	0.045	0.99	83.81	3.16	0.88
A. fumigatus	526	0.028	0.99	13.52	1.60	0.98

Conclusion

The BPB dye uptake ability of the biomasses of four isolated fungal strains (*R. stolonifer*, *Fusarium* sp., *Geotrichum sp.*, and *A. fumigatus*) was evaluated. The results showed that the biosorption of BPB dye was dependent on the pH of the dye solution. Higher capacities were obtained at pH 2.0 for all the biomasses. The four fungal stains presented high efficiency for BPB dye removal, especially the biomass of *R. stolonifer,* which was capable of removing more than 88% of coloring material up to an initial dye concentration of 800 mg/L. The kinetic studies revealed that the biosorption processes agreed well with the second-order adsorption kinetics model. In addition, the Langmuir model was found most suitable for describing the biosorption of BPB dye in the concentration range studied. The results suggest that these fungal biomasses were suitable for the development of efficient biosorbent materials for the decolorization of dye wastewaters.

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