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BIOSORPTION OF PHENOL FROM AQUEOUS SOLUTIONS BY FUNGAL BIOMASS OF *PHANEROCHAETE CHRYSOSPORIUM*

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ABSTRACT. The biosorption of phenol from aqueous solution on non-living mycelial pellets of *Phanerochaete chrysosporium* was studied using batch technique with respect to pH, initial concentration and biomass dosage. *Ph. chrysosporium* was grown in a liquid medium with a simple constitution. The phenol biosorption studies on fungal biomass was carried out at an initial pH of 5. Adsorption kinetics was characterized at an initial concentration of 12.5, 25 and 50 mg/L in a suspension concentration of 5.0 g/L. The sorption process followed the second-order kinetics. Phenol adsorption isotherms were determinated on fungal biomass at biomass concentration of 1.0 and 5.0 g/L and initial pH of 5. The adsorption equilibrium of phenol from aqueous solutions by mycelial pellets could be well described with Freundlich equation. The adsorption capacity of phenol and the Freundlich constant decreased with increasing biomass concentration.

Keywords: *phenol, biosorption, Phanerochaete chrysosporium biomass, adsorption isotherm, adsorption kinetics, water treatment*

INTRODUCTION

Wastewaters containing phenolic compounds present a serious problem. Wastewaters containing phenol cannot be discharged in surface waters without prior treatment due to the phenol toxicity. The toxic and hazardous nature of phenols and their associated derivatives, and their increasing amounts in industrial wastewaters have been documented. They are known human carcinogens. Phenolic compounds are present in the wastewaters generated by paint, solvent, petroleum, coal-conversion, pharmaceutical, wood preserving chemicals, plastic, rubber-proofing, pesticide, iron-steel, paper and pulp industries.

Traditionally, adsorption on activated carbon and polymer based adsorbents is the most widely used technique for the removal of phenols. The high cost of activated carbon and polymer has stimulated interest to use cheaper

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raw environmental-friendly materials as adsorbents. Recently, microorganisms have been considered as one of the most promising adsorbents [1-12]. However, information on fungal interacting with toxic phenolic compounds is still limited. In the concept of biosorption, several chemical processes may be involved, such as adsorption, ion exchange, and covalent binding. The biosorptive sites on the microorganisms are carboxyl, hydroxyl, sulphuryl, amino and phosphate groups [4,5]. Fungal cell walls and their components have a major role in biosorption. Aksu and Yener evaluated the biosorption of phenol and monochlorinated phenols on the dried activated sludge [6]. Ning et al. reported that anaerobic biosorption of 2,4-dichlorophenol was mainly a physicochemical process. They studied the equilibrium sorption isotherms and sorption kinetics of 2,4-DCP on live and chemically inactivated anaerobic biomass [7]. Rao and Viraraghavan have used nonviable pretreated cells of *Aspergillus niger* to remove phenol from an aqueous solution, and observed that maximum removal of phenol occurred at an initial pH of 5.1 [8]. Other workers investigated the biosorption capacity of dead and live fungal biomass, and they found, that better removal was achieved with dead fungal biomass than with live one [9-11].

Wu and Yu have used *Phanerochaete chrysosporium* biomass as a sorbent material for removal of phenol and chlorophenols [12,13]. They found that the sorption capacity on mycelial pellets increased in order: phenol < 2-CP < 4-CP < 2,4-CP. The adsorption increased with decreasing water solubility and increasing octanol-water partitioning coefficients. The presence of 2-CP or 4-CP and the initial concentration of 2-CP and 4-CP had no significant effect on the sorption of 2,4-DCP on fungal mycelial pellets. These suggests that partitioning was largely involved in biosorption mechnisms, and that hydrophobicity might govern the biosorption of phenolic compounds by mycelial pellets [4,12,13].

The objectives of this study were:

1. to test the biomass of *Phanerochaete chrysosporium* grown in a medium having simple constitution for phenol biosorption,
2. to evaluate the influences of different experimental parameters on biosorption such as initial pH, sorption time and initial phenol concentration using batch technique,
2 to model phenol biosorption kinetics by mycelial pellets using pseudo-first-order and second-order kinetic equations,
3 to determine adsorption isotherms using batch technique and analyse the adsorption equilibrium using Freundlich-equation,
4 to investigate the effect of biomass concentration on biosorption process.

RESULTS AND DISCUSSION

Factors influencing biosorption of phenol by mycelial pellets

Effect of initial pH on phenol biosorption on *Phanerochaete chrysosporium* in aqueous suspension

The effect of initial pH on the equilibrium uptake capacity of phenol by mycelial pellets of *P. chrysosporium* at pH values between 3.0 and 10.0 and 22.5 ± 2 °C is shown in Figure 1. The biosorption of fungi was influenced by pH in a range of 3.0 – 10.0. The initial concentration of phenol was 25 mg/L and the biomass concentration was 0.5 g/L. The maximal adsorbed phenol amount was $q_{\max} =$ 0.073 mg/g at pH 4. In the natural state of phenol solution, pH 5.0, without pH adjustment, the adsorption was slightly reduced, the adsorbed amount of phenol was 0.065 mg/g. The adsorption was reduced in an alkaline medium and slightly reduces in an acidic medium. The effect of pH on the adsorption of phenol was not significant at pH 3.0 – 6.0, and the uptake of phenol in the same pH interval was larger than that observed at other pH values. Further biosorption experiments were carried out at the natural state of pH 5 in the biomass suspensions.

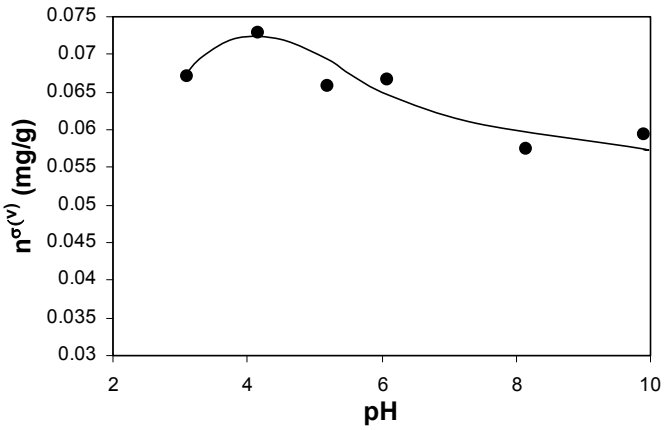

Figure 1. The pH effect over the phenol biosorption on *Phanerochaete chrysosporium* biomass. The biomass concentration is 0.5 g/L and the initial phenol concentration is 25 mg/L.

Phenol is weakly acidic, and pH has a significant effect on the degree of ionization of phenol and the cell surface properties. The amount of adsorbed phenol seemed to be related to the dissociation constant (pK_a), which is 9.9 for phenol [14]. The ionic fraction of phenolate ion increases which increasing pH, and phenol could be expected to become more negatively charged as pH increases. The surface charge on fungal biomass

is predominately negative at pH 3.0 – 10.0 [8, 13]. At pH < 3.0, the overall surface charge on fungal cells become positive. Thus, phenol can be adsorbed to a lesser extent at pH ≥ pK_a , due to the repulsive forces prevailing at higher pH values. A lower pH value resulted in a higher undissociated fraction of phenol and led to a decrease in phenol uptake by the mycelium pellets.

Adsorption kinetics of phenol on *Phanerochaete chrysosporium* biomass in aqueous suspension

The phenol adsorption kinetics was investigated on the biomass at a suspension concentration of 0.5 g/L. The initial phenol concentrations were of 12.5; 25 and 50 mg/L. In the figure 2 the adsorbed phenol amounts are presented against the adsorption time. The results show that adsorption equilibrium was reached during sixty minutes. The adsorption rate was higher in the first thirty minutes, but decreased until the equilibrium was reached. Similar trends were found by other workers [2, 12, 13, 15]. It should be noticed that the adsorption of phenol increased with an increas of the sorption time.

In the adsorption equilibrium at initial phenol concentration of 12.5 mg/L the maximal adsorbed phenol amount is q_{max} = 0.09 mg/g, at initial phenol concentration of 25.0 mg/L the maximal adsorbed phenol amount is q_{max} = 0.10 mg/g, and at initial phenol concentration of 50.0 mg/L the maximal adsorbed phenol amount is q_{max} = 0.19 mg/g.

To evaluate the biosorption kinetics of phenol, two kinetic models were used to fit the experimental data at different initial concentrations at pH 5.0.

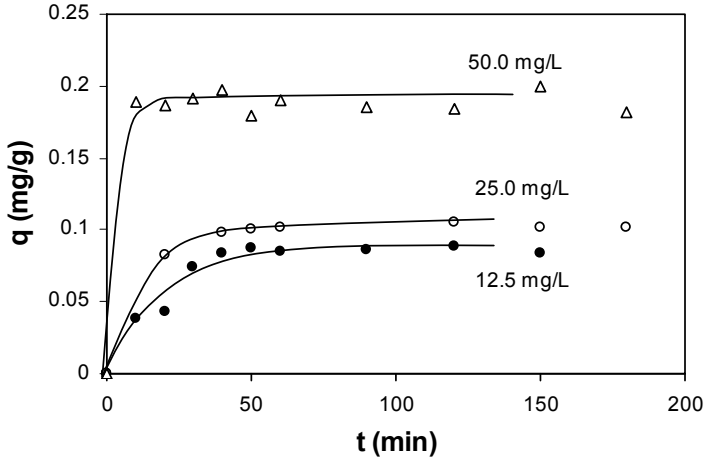

Figure 2. The effect of initial concentration on the sorption kinetics of phenol by mycelial pellets of *Phanerochaete chrysosporium*, initial concentration: 12.5; 25.0; 50.0 mg/L, temperature: 22.5 ^{o}C, biomass concentration: 5 g/L.

Pseud-first-order Lagergren model

The pseudo first-order rate expression of Lagergren model [16] is generally expressed as follows:

$$\frac{dq}{dt} = k_{1,ad}(q_{eq} - q) \tag{2}$$

where,

q_{eq} and q have their usual meanings and

$k_{1,ad}$ is the rate constant of first-order biosorption (min^{-1}).

The integrated form of equation (2) is:

$$\log(q_{eq} - q) = \log q_{eq} - k_{1,ad}\frac{t}{2.303} \tag{3}$$

However, to fit equation (3) to experimental data, the value of q_{eq} (equilibrium sorption capacity) must be pre-estimated by extrapolating the experimental data to t = ∞. In addition, in most cases the first-order rate equation is usually applicable over the initial 30 – 50 minutes of the sorption [13, 17, 18]. The plots of $\log(q_{eq} - q)$ as a function of sorption time are shown in Figure 3a. The linear relationships were observed only for the initial 60 minutes of sorption and the experimental data considerably deviated from the theoretical ones (not shown in the figure) after this period. The rate constants $k_{1,ad}$ and theoretical values of q_{eq} calculated from the slope and intercept of the linear plots are summarized in table 1 along with the corresponding correlation coefficients. The first-order rate constants $k_{1,ad}$ and the equilibrium sorption capacities $q_{eq,cal}$ ($q_{eq,cal}$ = 0.103 mg/g for the initial concentration of 12.5 mg/L, $q_{eq,cal}$ = 0.104 mg/g for 50.0 mg/L) have almost the same values for both initial concentration of 12.5 and 25.0 mg/L. In the case of initial concentration of 50.0 mg/L acceptable calculated results were not received using the first-order Lagergren model.

Pseudo- second-order kinetic model

If the sorption rate is second-order, the pseudo second-order kinetic rate equation is expressed as [18]:

$$\frac{dq}{dt} = k_{2,ad}(q_{eq} - q)^2 \tag{4}$$

where,

$k_{2,ad}$ is the rate constant of second-order biosorption (g/mg min) After integration, the following equation is obtained:

$$\frac{t}{q} = \frac{1}{k_{2,ad}q_{eq}^2} + \frac{t}{q_{eq}} \tag{5}$$

It should be noticed that for the utilization of this model, the experimental value of q_{eq} is not necessary to be pre-estimated. By plotting t/q against t for the initial concentrations (12.5, 25.0, 50.0 mg/L), straight lines were obtained as shown in Figure 3b. The second-order rate constants $k_{2,ad}$ and q_{eq} values are presented in table 1 were determined from the slopes and intercepts of the plots. The results show that the second-order rate constants $k_{2,ad}$ increased with an increase in initial phenol concentration. The biosorption of 2,4-dichlorophenol from aqueous solution on non-living pellets of *Phanerochaete chrysosporium* grown by Kirk et al [20] was also studied by Wu and Yu [12,13]. On the basis of their experiments they also found that the biosorption process to biomass followed pseudo second-order kinetics. The pseudo-second-order kinetic constants decreased with an increase in initial concentration for 2,4-dichlorophenol biosorption [13].

The correlation cefficients for the second-order kinetic model were close to 1.0, and the theoretical values of q_{eq} also agreed well with the experimental data. On the other hand, the correlation coefficients for the pseudo-first-order kinetics were lower than those for the pseudo-second-order one. Using pseudo-first-order model the theoretical q_{eq} values did not give reasonable values. This can be explained that the sorption of phenol on mycelial pellets follow the second-order kinetics. The second-order kinetic parameters can be used to determine the equilibrium sorption capacity, percent of the removal of phenol, rate constants and initial sorption rate for a bioreactor design.

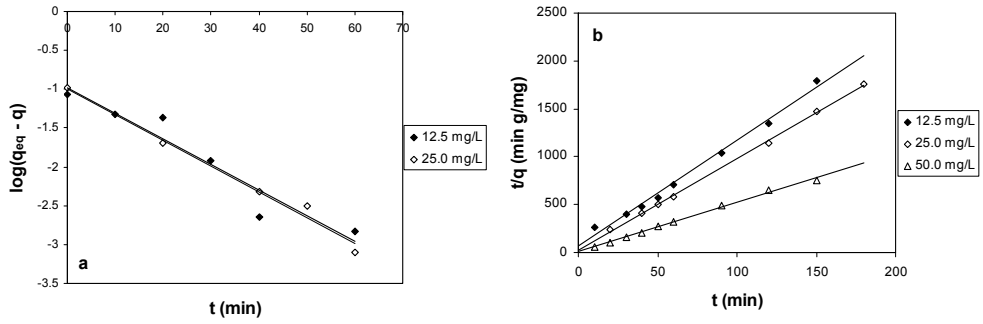

Figure 3. **(a)** Linearized pseudo-first-order kinetic model for phenol by mycelial pellets of *Phanerochaete chrysosporium* at different initial concentrations, initial concentrations: 12.5 and 25.0 mg/L, temperature: 22.5 °C, biomass concentration: 5 g/L.
(b) Linearized pseudo-second-order kinetic model for phenol by mycelialpellets of *Phanerochaete chrysosporium* at different initial concentrations, initial concentrations: 12.5, 25.0, 50.0 mg/L, temperature: 22.5 °C, biomass concentration: 5 g/L.

Table 1. The first-order and secon- order adsorption rate constants of phenol for different initial concentrations, at pH 5.0, temperature: 22.5 °C, biomass concentration: 5 g/L

c_0 (mg/L)	$k_{1,ad}$, (min^{-1})	$q_{eq,cal}$ (mg/g)	R^2	$k_{2,ad}$ (g/mg min)	$q_{eq,cal}$ (mg/g)	R^2	$q_{eq,exp}$ (mg/g)
12.5	0.076	0.104	0.918	1.861	0.090	0.991	0.086
25.0	0.077	0.103	0.985	3.475	0.105	0.999	0.103
50.0	-	-	-	4.172	0.193	0.997	0.189

Adsorption isotherms of phenol by *Phanerochaete chrysosporium* biomass in aqueous suspension

The biosorption isotherms of phenol by mycelium pellets were evaluated in the initial concentration range of 10 – 100 mg/L by varying biomass dosage. The biomass concentrations were 1.0 and 5.0 g/L. It is observed from Figure 4a that the uptake of phenol by biomass increases with the decrease of the biosorbent dosage and also increases with an increase of the initial concentration of phenol in solution. When suspension concentration was 5 g/L the maximal adsorbed amount was about 0.3 mg/g, while in the suspension of concentration of 1 g/L it was about 0.9 mg/g.

Analysis of equilibrium is important for developing a model that can be used for the design of biosorption systems. Two classical adsorption models, Langmuir and Freundlich isotherms, are mostly frequently employed.

Freundlich isotherm

The Freundlich equation based on sorption on a heterogeneous surface is given below as equation (6):

$$q_{eq} = K_F C_{eq}^{1/n} \tag{5}$$

where,

K_F and n are the Freundlich constants, which are indicators of adsorption capacity and adsorption intensity of the sorbents [6, 7, 21]. Equation (5) can be linearized in logarithmic form as follows:

$$\log q_{eq} = \log K_F + \frac{1}{n} \log C_{eq} \tag{6}$$

The values of K_F and n can be estimated respectively from the intercept and slope of a linear plot of experimental data of log q_{eq} versus log C_{eq}.

The linearized Freundlich adsorption isotherms of phenol obtained using different biomass dosages are shown in figure 4.b. The values of K_F and n calculated from the plot are also given in table 2. along with the regression correlation coefficients. The parameter K_F related to the sorption capacity increased with decreasing biomass concentration and thus increasing

maximal adsorbed amount of phenol. In table 2, n is less than unity, indicating that phenol is slightly linearly adsorbed by mycelial pellets in the equilibrated concentration range of 10 – 90 mg/L at temperature of 22.5 °C, and then the monolayer is saturated. Adsorption equilibriums of organic pollutants, such es phenol and chlorophenols, followed the Freundlich isotherm better than the Langmuir one [13, 15, 21].

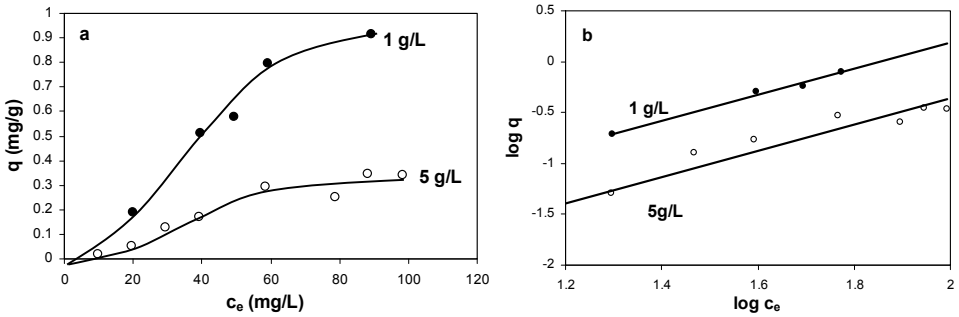

Figure 4. (a) Phenol adsorption isotherms by mycelial pellets of *Phanerochaete chrysosporium* from aqueous solutions at the biomass concentrations of 1 g/L and 5 g/L in the initial concentration range of 10 – 100 mg/L.
(b) Linearized adsorption isotherms of Freundlich.

Table 2. The Freundlich isotherm constants of phenol on micelial pellets in the initial concentration range of 10 –100 mg/L, at different biomass concentrations, at pH of 5.0

biomass dosage (g/L)	q_{eq} (mg/g)	K_F (mg/g)(mg/L)^n	n	R^2
1.0	0.9	0.004	0.78	0.989
5.0	0.3	0.001	0.76	0.942

CONCLUSIONS

The potential of non-living mycelial pellets of *Phanerochaete chrysosporium* grown in a medium of simple constitution to adsorb phenol molecules from aqueous solution was demonstrated in this study. The sorption capacity increased with an increase in initial phenol concentration and decreased with increasing biomass concentration. The biosorption of phenol by *Ph. chrysosporium* followed pseudo-second-order kinetics. The second-order kinetic constants increased with increasing initial concentration. The Freundlich model exhibited a good fit to the adsorption data of phenol.

In the studied concentration range of 10 – 100 mg/L phenol was adsorbed almost linearly by the fungal biomass and then the monolayer coverage was reached. Increasing biomass concentration, the maximal adsorbed amount of phenol decreased, and thus the calculated Freundlich constant decreased as well.

EXPERIMENTAL SECTION

Microorganism and its growth conditions

Phanerochaete chrysosporium a white-rot fungus, obtained from the Institute of Microbiology, University of Pécs, was maintained by subculturing on potato dextrose agar slants. Hyphal suspensions were prepared from 7-day old cultures, grown on potato dextrose agar slants at 35 ± 2 °C. *Ph. chrysosporium* was grown in a liquid medium containing (g/L) D-glucose, 10.0; KH_2PO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.5; NH_4Cl, 0.12; $CaCl_2 \cdot H_2O$, 0.1; thiamine, 0.001. The medium pH was adjusted to 4.5 with 1.0 mol/L HCl and 1.0 mol/L NaOH. The incubation was carried out at 39 °C in an orbital shaker incubator at 150 rpm for 5 days [1].

Preparation of the biosorbent

After 5 days, the mycelial pellets were harvested through filtering. The biomass was then washed thoroughly with distilled water to remove the growth medium adhering on its surface. In order to exclude the possibility of biodegradation of phenol by living mycelia, the mycelial pellets used in the all adsorption experiments were inactivated at 120 °C and 104 kPa for 20 min. The biosorbent used in this study was in the form of mycelium pellets without homogenization. Therefore, term particle size reffers to the diameter of the mycelial pellet.

Chemicals

Phenol (>99 % purity) was purchased from Sigma-Aldrich Ltd (Hungary) and was used without further purification. All other inorganic chemicals were of analytical grade. Stock solutions were prepared by dissolving 0.1 g of phenol in 1.0 L of distilled water. The test solutions containing phenol were prepared by diluting 100 mg/L of stock solutions of phenol to the desired concentrations. The phenol concentrations of prepared solutions varied between 10 – 100 mg/L in the sorption experiments. The pH value of the solutions in this study (2.0 – 11.0) was adjusted to the required value by using NaOH or HCl solutions. All solutions were stored in the dark at 4 °C prior to use.

Batch experiments

Biosorption experiments were carried out in batch mode. The biomass concentration was 5.0 g/L, (0.25 g dry mycelial pellets mixed with 50 mL of solution containing a pre-determined concentration of phenol). Mycelial pellets and phenol solution were placed in a test-tube, which was subsequently

covered to prevent photodegradation. All adsorption experiments were conducted in the dark to avoid formation of photodegradation products. Tubes were agitated on a shaker at 150 rpm and a constant temperature (22.5 ± 2 °C). Samples were taken at given time intervals, and then centrifuged at 10 000 rpm for 10 min. The supernatant was used for analysis of the residual phenol. The amount of phenol adsorbed at equilibrium, q (mg/g), was obtained as follows:

$$q = \frac{(c_0 - c_e)V}{m} \quad (1)$$

where,

c_0 and c_e are the initial and equilibrium liquid phase concentrations (mg/L)
V is the volume of the solution (L) and
m is the weight of the dry biomass used (g).

Analysis

Phenol concentration in supernatant was determined by HPLC. The HPLC system containes a liquid chromatograph (LC-10 AD_{VP}, Shimadzu), a micro vacuum degasser (DGU-14 A, Shimadzu), a system controller (SCL-10 A*VP*, Shimadzu), a diode array detector (SPD-M 10 A*VP,* Shimadzu) and an injector (7725i, Rheodyne). The LCMS solution software was applied on the HPLC system. The measurements were performed on the UV/VIS-photo diode array detector with detection at 270 nm. Chromatographic separations were performed on a Phenomenex C18 column (150×4.6 mm i.d., 5 μm, Phenomenex, USA). For separations the mobile phase A, water and mobile phase B, methanol and gradient system (0.03 min 42 % B eluent, 8.00 min 60 % B eluent, 8.10 min 42 % B eluent and 11.00 min 42 % B eluent) were used. Operating conditions were as follows: flow rate 1.0 mL/min, column temperature ambient and injection volumes 20 μL of the standard and samples. Calibration curve of the standard was made by diluting stock solution of standard in water to yield 10-100 mg/L.

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