Removal of humic substances by biosorption

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Abstract

Fungal pellets of Aspergillus niger 405, Aspergillus ustus 326, and Stachybotrys sp. 1103 were used for the removal of humic substances from aqueous solutions. Batchwise biosorption, carried out at pH 6 and 25 °C, was monitored spectrophotometrically and the process described with Freundlich’s model. Calculated sorption coefficients $K_f$ and $n$ showed that A. niger exhibited the highest efficiency. A good match between the model and experimental data and a high correlation coefficient ($R^2$) pointed out to judicious choice of the mechanism for removal of humic substances from the reaction medium. The sorption rate constants ($k$) for A. ustus and Stachybotrys sp. were almost equal, however higher than that for A. niger. Comparison of test results with the simulated ones demonstrated the applicability of the designed kinetic model for removal of humic substances from natural water by biosorption with fungal pellets. Different morphological structure of the examined fungal pellets showed that faster sorption does not imply the most efficient removal of humic substances. Desorption of humic substances from fungal pellets was complete, rapid, and yielded uniform results.

Key words: biosorption; humic substances; fungal pellets; aqueous solution; desorption

Introduction

Biosorption is a promising method for removal of various organic and inorganic impurities from natural water and dissolved solutions. The term “biosorption” refers to the passive uptake of pollutants from aqueous solutions by the use of non-growing or non-living microbial mass and indicate a number of metabolism-independent processes (physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation, and microprecipitation) taking place essentially in the cell wall (O’Neill et al., 1999; Koopal et al., 2001; Aksu, 2005). In this process, living microorganisms and biopreparations play active and passive roles respectively. Humic substances (HS) can be removed from natural water with physicochemical methods as well as with biological methods that employ microorganisms, i.e., by biosorption (Zhou and Banks, 1992; Schwarzenbach and Gschwend, 1993). Biochemical and morphological characteristics of microorganisms determine what mechanism will be applied to remove humic substances, organic compounds and metals (Zhou and Banks, 1991). The process itself comprises simple physical-chemical binding to a specific site on the cellular or extracellular molecules and metabolic intracellular transport of substances (Zhou, 1999). To date, research has been done on the same (Briški et al., 1994; Vuković et al., 1999) or related (Petrović et al., 1993; Ortego-Calvo and Saiz-Jimenez, 1998) fungal species, where the substrate was a humic substance or some other organic compound in the aqueous solution or some other organic compounds (Grebil et al., 2001).

Humic substances are indispensable for microbiological processes in many eco-systems. HS make half of the total organic carbon dissolved in water and are, therefore, subject to much research work. The structure has been elucidated of only 10%–20% of water-dissolved organic carbon. However, the content of fatty acids, amino acids and carbohydrates in natural water is known. The remaining dissolved organic carbon is not easy to define. Therefore, a common term “humic substances” has been adopted for the substances generated by natural degradation and decomposition of biological materials, plants or organic substances in soil and water (Thurman, 1986; Schulten and Gleixner, 1999).

Humic substances have become increasingly important with regard to water supplies, because it reacts with chlorine during the disinfection process in drinking water treatment and produces disinfection by-products such as trihalomethanes (THMs). The formation of THMs in drinking water is of much concern because of their carcinogenic effects on humans. THMs constitute a potential health risk (Imai et al., 2003).

The aim of the present work was to elucidate the role of three fungal species, Aspergillus niger, Aspergillus ustus...
and Stachybotrys sp., in the removal of HS from aqueous solution. Kinetic parameters necessary for quantitative comparison of their efficiency under given conditions were assessed by experiment, selection of a kinetic model and mathematical modeling. The Freundlich sorption model was used for determining the biosorption rates of HS onto fungal pellets. The effect of biosorption HS on fungal species was applied for investigations of the kinetics of the desorption process.

1 Materials and methods

1.1 Humic substances

HS used in the present work were isolated from ground water (Ivanić Grad, Croatia) according to the conventional procedures by NaOH dissolving and HCl precipitation (Petrović et al., 1999). Stock solution of HS was prepared by dissolving 1.0 g of a solid HS into a 1-L deionized water and filtered through a 0.45-µm membrane filter (Sartorius, Germany). Aqueous solutions of HS (5, 10, 15, 20, and 40 mg/L) were prepared by diluting stock solution in deionized water.

1.2 Fungi

The mold Aspergillus niger (FKIT 405) was from the Faculty of Chemical Engineering and Technology (FKIT) Culture Collection. The molds Aspergillus ustus (FKIT 326) and Stachybotrys sp. (FKIT 1103) were isolated from a water suspension after backwashing the filter medium (pilot plant for HS removal from groundwater in Ivanić Grad, Croatia). The fungi were isolated from the sample of well-homogenized aqueous suspension using malt agar medium (Biolife Manual, 1991) over which sterile oxytetracycline (2 ml of 1% antibiotic solution to 100 ml of medium) was added to suppress bacterial growth. Incubation at 28°C lasted over 3–5 d. Pure cultures of the fungi isolates were made and transferred onto malt agar slants as stock cultures. The microscopic (Olympus BX50, digital camera DP 10, Japan) and macroscopic features of the hyphal mass, morphology of cells and spores, and nature of the fruiting bodies were used for identification (Smith, 1969). Cultures were maintained on malt agar slants and subcultured every month, then transferred to storage at 4°C.

1.3 Sorbents

The sorbents were the pellets of A. niger (FKIT 405), A. ustus (FKIT 326), and Stachybotrys sp. (FKIT 1103). The pellets were cultivated under the submerged condition in 500 ml Erlenmeyer flasks with 100 ml of potato medium each, kept over 3 d in the rotary shaker at 160 r/min, pH 6.7 and 25°C. Potato medium (DSM Catalogue of Strains, 1989) was inoculated with the spore suspension at a final density of 7 × 10^6 spores/ml. The fungal pellets that developed were then harvested over a nylon filter (20 mesh) washed five times with sterile deionized water, rinsed with phosphate buffer (pH 7.2) and kept in the refrigerator at 4°C until further use.

1.4 Biosorption

Biosorption studies were conducted in a glass column (500 ml) using 300 ml of humic substances aqueous solution (5–40 mg/L) and 3.0 g of fungal pellets (wet weight) at pH 6.0 and 25°C. Continuous air flow of 1 L/h was maintained with a membrane pump HI-TOP 1500 through a rotameter (0.05–0.5 L/min, Cole-Parmer, USA) to ensure mixing of the reaction medium. Controls were run with a blank HS solution system. Samples were taken every 30 min and filtered immediately through a 0.45-µm membrane filter. The filtrate was analyzed for residual HS concentration in solution until sorption equilibrium was reached (Sag et al., 2000). The concentrations of HS in these phases were measured at λ = 340 nm by spectrophotometer MA-9510 (Iskra, Horjul, Slovenia). All experiments were carried out in triplicate. Biosorbed HS concentrations were the means of the three replicates. Concentrations of HS sorbed on the fungal pellets (q, mg/g) were expressed per unit of wet biomass, according to Eq.(1):

\[ q = \frac{(c_0 - c) \times V}{M} \]  

where, \( c_0 \) (mg/L) and \( c \) (mg/L) is HS concentration in the solution at the beginning and the end of biosorption, respectively. \( V \) (L) is the volume of HS solution and \( M \) (g) is mass of a wet biomass.

1.5 Desorption

After biosorption, the reaction medium was passed through a 0.45-µm membrane filter. The pellets of A. niger, A. ustus, and Stachybotrys sp. were washed with sterile deionized water. The washing steps were repeated five times and dewatered biomass weighed (Briški et al., 1994). Desorption studies were conducted in 250 ml Erlenmeyer flasks using 50 ml of 0.1 mol/L NaOH and 1 g of the dewatered fungal pellets (wet weigh). The flasks were agitated on a rotary shaker at 160 r/min and 25°C. Concentration of desorbed HS was measured as described above.

1.6 Data assessment

Model parameters were assessed by linear analysis using a least squares method, as a part of “MS Excel” program package. For non-linear regression analysis and optimization of model parameters the “MS Solver” program package was applied (Sag et al., 2000). Differential equations of the model were numerically solved, applying Euler’s algorithm (Rice and Do, 1995). A group of the model’s optimal parameters was used for simulations that were compared with the experimental results.

2 Results and discussion

2.1 Biosorption

Property of sorbents and concentration of sorbate in solution affect the biosorption of humic substances from aqueous solution. With reference to similar studies by Briški et al. (1994), biosorption was performed with fungal
pellets of A. niger 405, from the Culture Collection FKIT, whereas A. ustus 326 and Stachybotrys sp. 1103 were isolated from water suspension. Growth conditions for fungal pellets were defined based on previous experimental findings (Vuković et al., 1999) and spherical pellets with an average diameter of 3.0 ± 0.5 mm were used for biosorption study. The morphological characteristics and size of fungal pellets were consistent with findings by El-Enshasy et al. (1999) and Li et al. (2000). Biosorption was batchwise under isothermal conditions and it was assumed that a contact time of 4 h was sufficient enough to attain equilibrium. Denizli et al. (2005) reported that biosorption of organic matter by fungal biomass also reached the equilibrium in 4 h. They also researched biosorption by increasing pH from 2.0 to 8.0 and found that the highest biosorption occurred under the optimal experimental conditions at pH 6 and at room temperature. It is in agreement with other researchers (Petrović et al., 1993; Briški et al., 1994; Lieávremont et al., 1998; Vuković et al., 1999), which performed biosorption under the same conditions.

The results of the biosorption of HS by fungal pellets of A. niger 405, A. ustus 326 and Stachybotrys sp. 1103 are presented in Fig.1. The results are expressed in the form of equilibrium adsorption isotherms, which show the distribution of HS between the biosorbent (fungal pellets) and aqueous solution at equilibrium. The equilibrium biosorption data were analyzed using the empirical Freundlich model (Freundlich, 1906; Esperza-Soto and Westerhoff, 2003):

\[ q_e = K_f \times c_e^n \]  

(2)

where, \( q_e \) (mg/g) is the equilibrium concentration of the HS sorbed on fungal pellets, \( c_e \) (mg/L) is the equilibrium concentration of HS in the solution, and \( K_f \) (L/g) and \( n \) are characteristic constants fitted to each series of data.

The Freundlich equation is frequently used to fit single-solute data. It is clear from Fig.1 that the Freundlich model provided a good fit at low concentrations (Zhou, 1999). This model described the experimentally observed data reasonably well. Table 1 summarizes the parameters for the Freundlich adsorption isotherm model. It was found that the values of sorption parameters were within the range for organic compounds (Lieávremont et al., 1998; Rao and Viraraghavan, 2002; Kleineidam et al., 2002).

Sorption occurred during intermediate transfer of the substances, between the mobile aqueous phase and immobile solid phase (sorbent). Eq.(3) is showing the rate of substance transfer, from the aqueous solution to sorbent (Altfelder et al., 2001):  

\[ r = k \times (K_f \times c_e^n - q) \]  

(3)

where, \( r \) (mg/(g·h)) is the rate of sorption on the sorbent, \( k \) (h\(^{-1}\)) is the sorption rate constant, and \( q \) (mg/g) is concentration of the sorbate on the sorbent. By studying process kinetics, applying the parameters optimization method, the constants of sorption rate, \( k \), and initial rates of humic substances sorption \( r \) were calculated with respect to the initial humic substances concentrations in the solutions.

The rate of sorption increased with increasing initial HS concentration in solution as presented in Fig.2. A. ustus exhibited the highest sorption rate (Fig.2 and Table 2). Biosorption itself was fast, with the equilibrium established in the first 3 h of the experiment. The correlation coefficients between the predicted and the experimental values for the entire data set range were 0.9987–0.9816.

Simulation (Fig.3) was performed based on the obtained parameters of the kinetic Freundlich sorption, Eq.(3).

Comparison of the results showed that the model

<table>
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<tr>
<th>Table 1</th>
<th>Freundlich constants and correlation coefficients for HS biosorption by fungal biomass</th>
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<tr>
<td>Fungi</td>
<td>( K_f ) (L/g)</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>3.20 ± 0.39</td>
</tr>
<tr>
<td>Aspergillus ustus</td>
<td>1.91 ± 0.21</td>
</tr>
<tr>
<td>Stachybotrys sp.</td>
<td>0.89 ± 0.08</td>
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![Fig. 1](image1.png)  
Fig. 1 Sorption isotherms of HS by different fungal species. Mean particle size is 3 mm.

![Fig. 2](image2.png)  
Fig. 2 Comparison of the effect of initial HS concentration on initial biosorption rates of HS by different fungal species.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Sorption rate constants and regression coefficients for the studied fungi</th>
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<tbody>
<tr>
<td>Fungi</td>
<td>( k ) (h(^{-1}))</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.1402 ± 0.008</td>
</tr>
<tr>
<td>Aspergillus ustus</td>
<td>0.1948 ± 0.009</td>
</tr>
<tr>
<td>Stachybotrys sp.</td>
<td>0.2011 ± 0.007</td>
</tr>
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</table>
Fig. 3 Comparison of the experimental results with biosorption simulation results for Aspergillus niger (a), Aspergillus ustus (b), and Stachybotrys sp. (c) regarding initial HS concentrations in the solution and model. (Eq.(3)), within the initial experimental concentrations, was capable of producing satisfactory dynamic picture of the change in concentration, i.e., humic substances removal from the aqueous solution at the process phase which is responsible for biosorption of fungal pellets.

During the short period (4 h) of biosorption, there were no changes in fungal pellets morphology. Before biosorption visually pellets were whitish while at the end of experiment they became dark brown. The morphology and structural density of fungal pellets depend on fungal species and the conditions of submerse growth. Photomicrographs (Fig.4) show the cross sections of fungal pellets, the structure of which is very important in humic substances removal. Biosorption causes HS incorporation into the inner part and external part of pellet structure (O’Neill et al., 1999). Dark coloration along the cross section of fungal pellets was an indicator of sorbed HS (Fig.4).

Figure 5 shows that A. niger exhibited the highest humic substances sorption in the concentration range 5–40 mg/L, which could be attributed to the structure of fungal pellets (Fig.4a). Results on biosorption content of HS are in agreement with other findings reported by Petrović et al. (1993).

The surface of the pellets of A. niger had loose hyphal networks that enabled sorption both in the exterior and the interior layers of a dense mycelial network. Pellets of fungus Stachybotrys sp. (Fig.4c), being structured fluffily and having markedly branched hyphae, had the fastest biosorption rate (Table 2). Less compact core region and external layer with peripheral hyphae is also a reason for more rapid sorption of pollutants by Stachybotrys sp. because of film diffusion and accessibility to active sites in the core region. In spite of that Stachybotrys sp. had the lowest HS sorption capacity $K_f = 0.89 \text{ L/g}$ in comparison with the other two fungi. It is supposed that those pellets possessed a smaller number of active sites. Another reason for different sorption rates despite of similar morphology of A. niger and Stachybotrys sp. is because of differences depending on the fungal genera and species. A. ustus pellets (Fig.4b) had a compact structure along the whole cross section, and its sorption, compared to A. niger, was faster. However, more rapid sorption did not ensure the most efficient removal of HS, as shown in Tables 1 and 2. The sorption capacity, $K_f$, of A. niger was 3.20 L/g despite a lower sorption rate ($k = 0.1402 \text{ h}^{-1}$). Calculated $K_f$ value is in agreement with other findings on biosorption by A. niger reported by Rao and Viraraghavan (2002).

Fig. 4 Photomicrographs of cross sections of the fungal pellets after biosorption. (a) Aspergillus niger; (b) Aspergillus ustus; (c) Stachybotrys sp. $P = 100 \times$. 

2.2 Desorption

Desorption of sorbed humic substances on fungal pellets was studied in batch system. Humic substances sorbed onto fungal pellets were eluted with 0.1 mol/L NaOH. Humic substances biosorbed on the pellets of all three fungi were fully desorbed. A desorption kinetic model, with the use of a linear isotherm (Rijnaarts et al., 1990), was expressed by Eq.(4):

$$-r_d = k_d \times (K_d \times c - q)$$  \hspace{1cm} (4)

where, $r_d$ (mg/g·h) is the desorption rate, $k_d$ (h⁻¹) is desorption rate constant, and $K_d$ (L/g) is distribution coefficient.

Constant $k_d$ was determined by linear regression analysis, using Eq.(4), and constant $K_d$ was determined by non-linear regression analysis (Rijnaarts et al., 1990; Haberhauer et al., 2000). The kinetic parameters of the mathematical models for all three fungi are shown in Table 3, and were within the range for organic compounds.

Table 3  Desorption rate constants, coefficients of distribution and regression for the studied fungi

<table>
<thead>
<tr>
<th>Fungi</th>
<th>$k_d$ (h⁻¹)</th>
<th>$K_d$ (L/g)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>3.5199 ± 0.12</td>
<td>0.93 ± 0.04</td>
<td>0.9984</td>
</tr>
<tr>
<td>Aspergillus ustus</td>
<td>2.9720 ± 0.09</td>
<td>0.92 ± 0.01</td>
<td>0.9958</td>
</tr>
<tr>
<td>Stachybotrys sp.</td>
<td>2.7546 ± 0.11</td>
<td>0.97 ± 0.06</td>
<td>0.9957</td>
</tr>
</tbody>
</table>

(Grebl et al., 2001; Kim and Kim, 2004). Desorption was rapid, about 1 h, and gave uniform results throughout the experiment as presented in Fig.6. Compared to A. ustus and Stachybotrys sp., A. niger had a superior desorption rate ($k_d = 3.5199$ h⁻¹).

3 Conclusions

The aim of this study was to find the biosorption characteristics of selected fungi. The results obtained showed that mycelial pellets of A. niger, A. ustus, and Stachybotrys sp. were good adsorbent media for humic substances and had high adsorption yields. The Freundlich adsorption model was used for the mathematical description of the biosorption of HS onto fungal pellets.

The process of biosorption was rapid and the equilibrium is established in the first 3–4 h of the experiment. The suitability of the kinetic models for the biosorption and desorption of humic substances were discussed. Freundlich isotherm has confirmed good agreement between the model and experimental data. A higher HS sorption rate was obtained with isolated fungi A. ustus and Stachybotrys sp. compared with A. niger. The different morphological structures of the used fungal pellets shows that more rapid sorption does not imply the most efficient removal of humic substances. Based on the experimental results it may be concluded that desorption of humic substances desorption from fungal pellets was complete, rapid, and yielded uniform results.

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