Sorption of Humic Acids onto Fungal Surfaces and Its Effect on Heavy Metal Mobility

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Abstract Mutual sorption interactions between heavy metals, humic acids and fungi were evaluated in this article. While the relative amount of sorbed As(V), Sb(III) and Pb(II) slightly decreased or remained unchanged, the sorption capacity of Zn(II) increased significantly with increasing amounts of immobilized humic acids in the Ca-alginate beads. Therefore, zinc is most likely preferentially sorbed to functional groups provided by humic acids rather than carboxyl or hydroxyl groups of alginate, with an optimum pH for uptake between 4 and 6. Nevertheless, the removal efficiency of metal(loid)s by unmodified Ca-alginate beads or those with humic acids modification was highest for Pb(II), at up to 93.5 %. The pH value also affects humic acids sorption properties on microbial surfaces. While the highest humic acids sorption capacity of mycelial pellets prepared from *Aspergillus niger* occurred at pH 8.5 (231 mg g^{-1}), the pelletized *Aspergillus clavatus* biomass was more effective in acidic solution and 199 mg g^{-1} was recorded there at pH 5.5. The effect of mutual interactions between humic acids and mycelial pellets on Zn(II) immobilization indicates that zinc affinity is higher for the fungal surface than for humic acids which do not supply sufficient active sorption sites for zinc. This resulted in less sorption capacity of the mycelial pellets modified with humic acids compared to the unmodified biomass.

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1 Introduction

Humic acids (HA) are complex natural acidic organic macromolecules with highly variable chemical composition and diverse active functional groups (Hladký et al. 2013). This enables intensive interaction with various soil phases (Arias et al. 2002) and soil organisms, including filamentous fungi (Vuković et al. 2008). Their exceptional sorption properties modulate the mobility of various elements in the natural environment, including potentially toxic metal(loid)s (Barančíková and Makovníková 2003). Therefore, humic acids can act as a natural barrier to limit organisms' sorption, uptake and accumulation of toxic metal(loid). Although mutual interactions between mineral phases, humic acids and toxic substances have been extensively studied in recent years (Badora 2012; Gardošová et al. 2011; Liu et al. 2011), the influence of humic acid-coated microbial biomass on the mobility of toxic metal(loid)s is omitted in literature.

The main object of this study is to evaluate the sorption properties of humic acids for the surfaces of two common fungal strains (*Aspergillus niger* and *Aspergillus clavatus*) and for the selected metal(loid)s (Pb(II), Zn(II), As(V) and Sb(III)). Concurrently, the effect of mutual interactions between humic acids and mycelia on immobilization of heavy metals is also examined.

2 Materials and Methods

2.1 Chemicals and Reagents

The sodium alginate and humic acids were purchased from Sigma-Aldrich Chemie (Germany). Stock solutions of As(V), Zn(II), Sb(III) and Pb(II) were prepared in deionized water by dissolution of $Na_2HAsO_4.7H_2O$ (Fisher Scientific, UK), $Zn(NO_3)_2.6H_2O$ (Slavus, Slovakia), $C_4H_4KSbO_7.0.5H_2O$ (Centralchem, Slovakia) and $Pb(NO_3)_2$ (Slavus, Slovakia).

2.2 Alginate Beads Preparation

Both pure alginate beads and beads with immobilized humic acids were prepared by dropping a 2 % (w/v) sodium alginate solution or a combined sodium alginate and dissolved humic acids solution (5–30 %) to a gently stirred 2 % (w/v) $CaCl_2$ solution at 25 °C. The cross-linking spherical products of both the pure Ca-alginate beads and those treated with immobilized humic acids had average diameter of approximately 2 mm. They were left in solution overnight at 4 °C, then collected by filtration and washed several times with distilled water and finally weighed for the following sorption experiments.

2.3 Sorption of Metal(loid)s onto Ca-alginate Beads with Immobilized Humic Acids

The metal(loid) stock solutions of Zn(II), Pb(II), As(V) and Sb(III) were diluted in distilled water to a final concentration of 500 mg L^{-1}, and 5 mL of this solution was added to 100 mL of distilled water and stirred at 120 rpm. This was done in 250 mL Erlenmeyer flasks with ≈0.08 g unmodified alginate beads or alginate beads modified with humic acids at 25 °C for 24 h, until equilibrium was reached. The samples were then filtered, and the total concentration of metal(loid)s was determined by FAAS (Perkin-Elmer 1100) in filtrate. The effect of pH on the sorption properties of humic acids immobilized in alginate beads for Zn(II) was then examined. After adding in 5 mL of Zn(II) solution, the pH was then adjusted from 3 to 9 by 1 M HCl or NaOH before the addition of modified or unmodified beads. All experiments were run in triplicate.

2.4 Biosorbent Preparation

Fungal strains of *A. clavatus* and *A. niger* were isolated from an indoor environment in Slovakia (Piecková and Jesenská 1998) and maintained on agar slants at 4 °C. Fungal conidia were harvested from 7-day old colonies cultivated at 25 °C on Sabouraud dextrose agar plates (HiMedia, India). The agar surface was rinsed in sterile deionised water, and 5 mL spore suspension diluted to approximately 10^6 CFU mL^{-1} was inoculated into 100 mL of culture medium (Sabouraud dextrose broth; Himedia, India) and incubated on a rotary Unimax 2010 shaker (Heidolph, Germany) in Erlenmeyer flasks at 120 rpm and 25 °C. Following 3-day incubation, the spherical mycelial pellet biomass was harvested by filtration and washed thoroughly in distilled water to remove any growth medium adhered to its surface.

2.5 Biosorption of Humic Acids onto the Fungal Biomass

Mycelial pellets of 10 g wet weight were mixed with 50 mL of 0.1 M phosphate buffer solution. The pH was adjusted to 5.5, 7 or 8.5 in 250 mL Erlenmeyer flasks, and 5 mL of stock humic acids solution with concentrations ranging from 50 to 450 mg L^{-1} was then added. Flasks were agitated on a rotary shaker at 120 rpm and 25 °C for a maximum of 20 h to ensure that sorption equilibrium was reached. After predetermined time period, the mixture was filtered through a 0.45 μm membrane filter and the residual concentration of humic acids in the filtrate was determined at 410 nm by SP-300 spectrophotometer (Optima, Japan). The wet humic acid modified fungal biomass was washed in a small amount of deionized water and used as biosorbent in following experiments. All sorption experiments were run in triplicate and processed in the dark to prevent humic acid photo degradation (Dziedzic et al. 2010). Finally, biomass without altered surface was prepared for control experiments.

2.6 Biosorption of Zn(II) onto HA Mycelial Pellets

Mycelial pellets with a known concentration of humic acids adsorbed onto their surface were used for experiments on Zn(II) biosorption. Individual flasks were placed on a rotary shaker at 120 rpm for 24 h. These contained 10 g of unmodified or modified wet biomass,

50 mL of distilled water and 5 mL of Zn(II) stock solution with initial concentrations of 5 to 20 mg L^{-1}. After membrane filtration, the residual content of Zn(II) in the filtrate was determined by ICP-OES on Jobin Yvon 70 Plus (Longjumeau, France). All experiments were run in triplicate, and control experiments were conducted without either biomass or Zn(II).

3 Results and Discussion

3.1 Metal(loid) Sorption onto Ca-alginate Beads with Immobilized Humic Acids

Removal efficiency of metal(loid)s by unmodified or humic acid modified Ca-alginate beads was highly effective for Pb(II) (Fig. 1b) while removal of zinc, arsenic and antimony was significantly lower (Fig. 1a). This difference in metal ion binding capacity may be due to both the metal(loid) and sorbent properties, including their structure and reactive sites (Arica et al. 2004). If we initially conclude that the increasing amount of humic

acids provide additional active sites for the sorption of studied elements, we would be correct, because humic acids are composed of the same functional group types (carboxylic and hydroxyl groups) responsible for alginate beads' removal of heavy metals (Kleinübing et al. 2011). However, when the sorption capacity of composite sorbent, expressed as the amount of metal(loid) immobilized by unitary sorbent mass was calculated as in Fig. 2, the relative amount of sorbed As(V), Sb(III) and Pb(II) slightly decreased or remained unchanged and showed a weak correlation with increasing concentration of immobilized humic acids in the alginate beads. Therefore, the increased metal(loid) removal efficiency in Fig. 1 should be attributed to an increase in the total mass of composite sorbent rather than to the direct effect of humic acids or their chemical composition. In general, the sorption capacities of the modified alginate beads for the examined metal(loid)s had the following order: Pb(II)>Zn(II)>Sb(III)>As(V). Similar behaviour to this has been reported by other authors (Li et al. 2010; Papageorgiou et al. 2006; Wu et al. 2012).

However, zinc behaved differently, and our experimental results clearly indicate that the sorption capacity increased with increasing amount of immobilized humic acids in the Ca-alginate beads (Fig. 2). This is attributed to the different removal mechanism of Zn(II) which preferentially binds to groups other than carboxylic or hydroxyl ones. Research by Kleinübing et al. (2013) indicates that the preferential sorption sites of zinc are sulphate and amino groups, which are also standard constituents of humic acids, while the pure alginate extract does not include these functional groups. Therefore, we concluded that zinc has the highest affinity for the humic acids in selected potentially toxic metal(loid)s. This conclusion was applied in the following experiments.
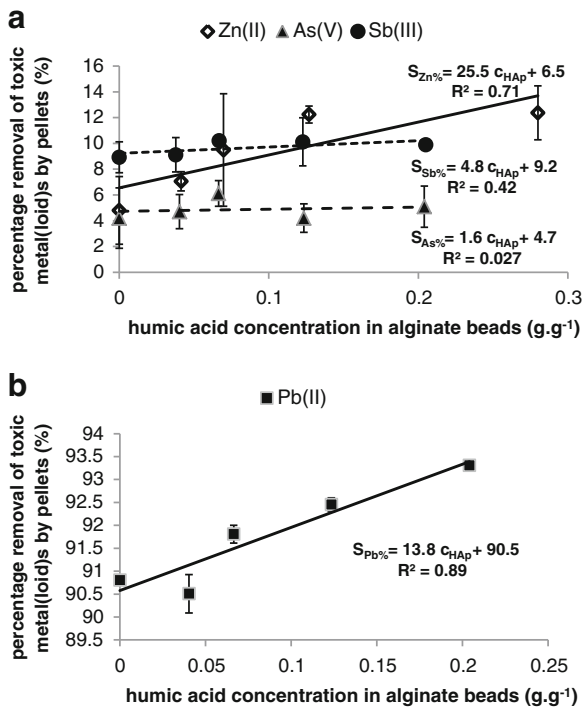
3.2 pH Influence on Zn(II) Sorption

The inflex point of Ca-alginate beads occurs at pH 3 to 4 (Veglio et al. 2002). Because of the significant protonation of functional groups responsible for Zn(II) binding, the affinity of Zn(II) to the alginate beads' surface is negligible at or below these pH values (Fig. 3a). However, when humic acids are introduced, the sorption capacity of composite material for zinc increased significantly in the examined pH range. This is due to both the different compositions of sorption sites in the composite sorbent and the different response of

Fig. 1 Metal(loid) removal efficiency ($S_\%$) at 120 rpm and 25 °C as affected by increased humic acids concentration (c_{HAp}) in the composite Ca-alginate sorbent. Initial concentration of each metal(loid) in water solution was 50 mg L^{-1}

Fig. 2 Influence of different humic acids content in Ca-alginate beads (c_{HAp}) on humic acids sorption capacity for metal(loid)s (S_c). Initial concentration of each metal(loid) was 50 mg L^{-1}
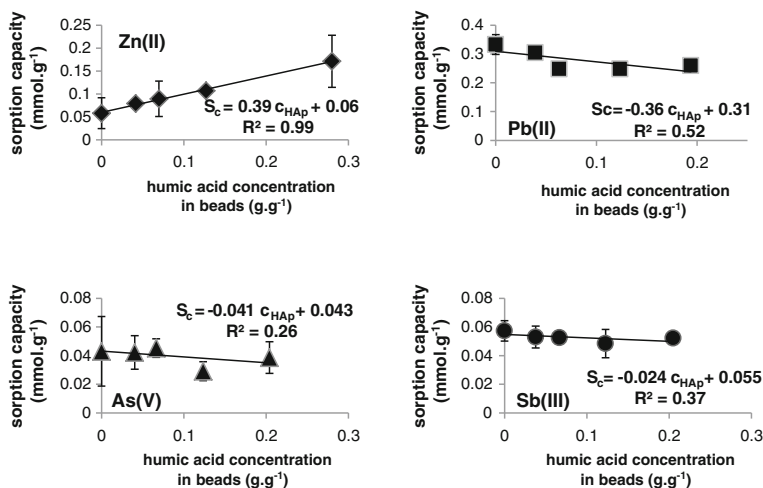
humic acids to pH (Gardošová et al. 2012). Although composite sorbent capacity increased steadily with increasing pH, our research data on the sorption capacity of sole humic acids suggested that the optimum pH for Zn(II) sorption lies between pH 4 and 6 (Fig. 3b).
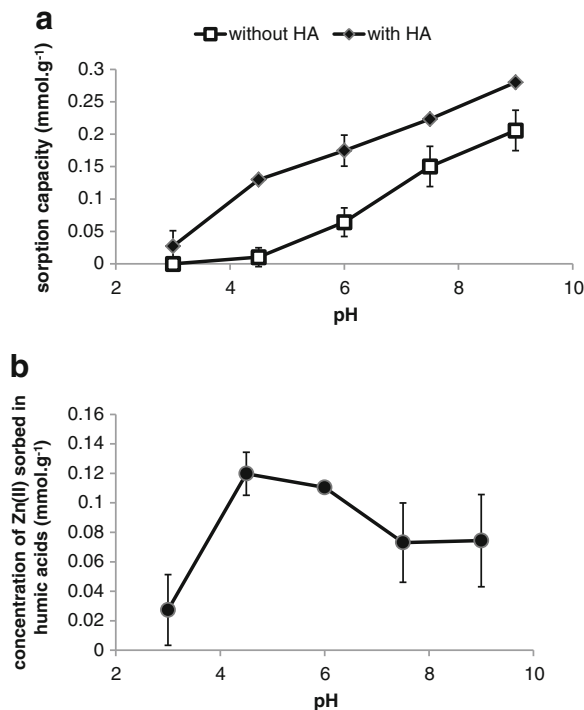
Fig. 3 **a** Zn(II) sorption from water solutions at different pH on pure alginate beads or those modified with humic acids and **b** sorption capacity of humic acids for zinc at pH's between 3 and 9. The concentration of humic acids immobilized in Ca-alginate beads was 0.03 g g^{-1}, and the initial zinc concentration for each treatment was 50 mg L^{-1}

3.3 Humic Acids Biosorption onto Mycelial Pellets

Equilibrium sorption experiments were conducted to evaluate the sorption capacity and affinity of humic acids for the surface of mycelial pellets prepared from *A. niger* and *A. clavatus* strains. Experimental data for humic acids sorption onto pelletized fungal biomass of *A. niger* and *A. clavatus* are presented as a functional dependence on the equilibrium concentration of humic acids in solution at three different pH's (Figs. 4 and 5). These data are described by Langmuir (**1**) and Freundlich (**2**) empirical isotherm models in the following equations:

$$S_{eq} = \frac{C_{eq} K_L S_{max}}{1 + K_L C_{eq}} \tag{1}$$
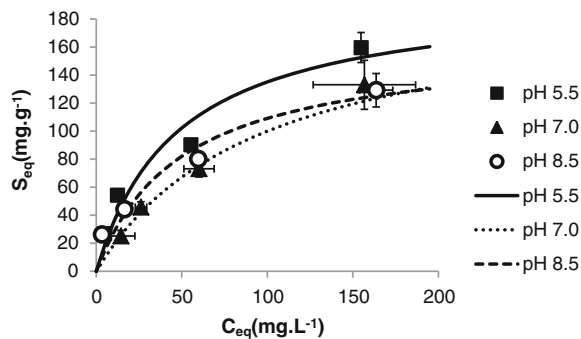
Fig. 4 Humic acids sorption by *Aspergillus clavatus* pelletized biomass fitted by the Langmuir isotherm (25 °C, 120 rpm and 20-h contact time)
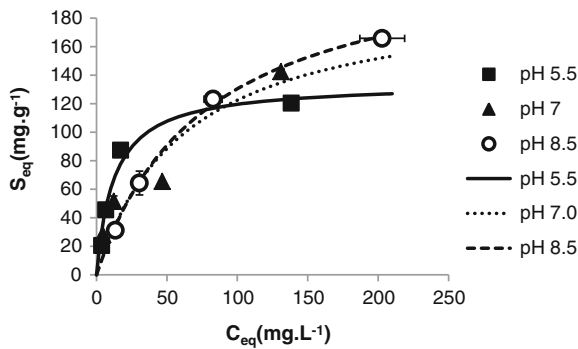
Fig. 5 Humic acids sorption by *Aspergillus niger* pelletized biomass fitted by the Langmuir isotherm (25 °C, 120 rpm and 20-h contact time)

$$S_{eq} = K_F C_{eq}^{1/n} \qquad (2)$$

where, S_{eq} is the humic acids uptake by biomass (mg g^{-1}), S_{max} represents the maximum humic acids uptake at defined pH and temperature (mg g^{-1}), C_{eq} is the equilibrium humic acids concentration in solution (mg L^{-1}), K_L (L mg^{-1}) is the Langmuir constant for sorbate to sorbent affinity, K_F is the Freundlich constant (L g^{-1}) related to sorption capacity of the biomass when the equilibrium concentration of sorbate in solution is unitary and n is the constant for binding site heterogeneity.

The Levenberg–Marquardt method was used to determine fitted parameters, and results were expressed by the Langmuir and Freundlich model with relatively good correlation coefficient values. These ranged from 0.84 to 0.99, thus indicating that both isotherms were suitable for data description. The values of both isotherm models constants and their respective correlation coefficient are presented in Tables 1 and 2.

Vuković et al. (2008) reported that the *A. niger* strain exhibited the highest humic acids sorption efficiency of all their applied fungal strains. Their quoted K_F

Table 1 Langmuir isotherm parameters calculated for humic acids sorption onto *Aspergillus clavatus* mycelial pellets

pH	Langmuir isotherm			Freundlich isotherm		
	K_L (L mg^{-1})	S_{max} (mg g^{-1})	R^2	K_F (L g^{-1})	n	R^2
5.5	0.02	199	0.94	14.0	2.0	0.98
7	0.01	195	0.84	8.6	1.9	0.84
8.5	0.02	163	0.93	12.9	2.2	0.98

Table 2 Langmuir isotherm parameters calculated for humic acids sorption onto *Aspergillus niger* mycelial pellets

pH	Langmuir isotherm			Freundlich isotherm		
	K_L (L mg^{-1})	S_{max} (mg g^{-1})	R^2	K_F (L g^{-1})	n	R^2
5.5	0.08	134	0.96	26.1	3.1	0.84
7	0.02	200	0.86	11.6	2.0	0.95
8.5	0.01	231	0.99	11.3	1.9	0.96

3.2 L g^{-1} value most likely results from *A. niger*'s loose hyphal network which enables sorption in both its exterior and interior mycelial layers. Our results show higher *A. niger* and *A. clavatus* sorption capacity for humic acids, with Freundlich constant values up to 26.1 L g^{-1}. However, despite the high correlation coefficient of the Freundlich isotherm fit, the unitary equilibrium concentration of humic acids in solution applied in the K_F calculation is far below the experimental equilibrium concentration range (Figs. 4 and 5). This renders it unsuitable for data interpretation, and the constants calculated from the Langmuir isotherm are more realistic.

Although the S_{max} constants of both *A. niger* and *A. clavatus* are relative similar, they differ slightly in response to pH, where the maximum sorption capacity of *A. niger* is higher under alkaline conditions (231 mg g^{-1}) and the removal efficiency of humic acids by *A. clavatus* is higher in acidic pH (199 mg g^{-1} at pH 5.5). This may be caused by differences in their functional groups responsible for humic acids sorption and the degree of surface ionization affecting microbial biomass sorption (Pagnanelli et al. 2003). Although the affinity of humic acids for *A. clavatus*'s mycelial surface expressed by the K_L Langmuir constant in Table 1 was
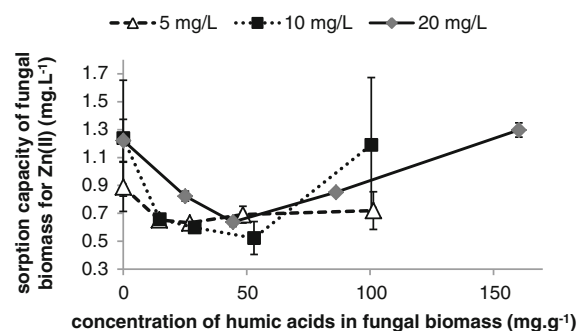

Fig. 6 Influence of humic acids concentration pre-adsorbed onto mycelial pellets surfaces on Zn(II) uptake by *A. niger* strain

not affected by pH changes, *A. niger*'s reaction of higher K_L values with decreasing pH indicated high removal efficiency of humic acids in diluted solutions (Table 2).

3.4 Zn(II) Biosorption onto Humic Acid Modified Mycelial Pellets

The efficient immobilization of heavy metals by mycelial surfaces (Jalili Seh-Bardan et al. 2013) can be significantly altered by the presence of other ions or molecules, including humic acids. Although humic acids adsorption onto mineral phase surfaces (Arias et al. 2002) or their presence in the adsorption system (Lai et al. 2002) enhanced the removal efficiency of bivalent heavy metals, the increasing amount of adsorbed humic acids on *A. niger*'s mycelial pellet surfaces affected the fungal sorption capacity for Zn(II) differently. Figure 6 highlights the same pattern for all initial Zn(II) concentrations, clearly indicating decreased sorption capacity for zinc compared to the unmodified control, especially when the sorbed humic acid concentration on the biomass is under 45 mg g^{-1}. Although the sorption capacity increases above this value, it was not significantly higher than the sorption capacity of native, unmodified mycelial pellets. These values were 0.89, 1.22 and 1.24 mg g^{-1} for initial zinc concentrations of 5, 10 and 20 mg L^{-1}, indicating surface saturation at higher Zn(II) concentrations. Therefore, we assume zinc affinity is higher for fungal surfaces than for humic acids which do not supply sufficient active sorption sites for zinc. Although fungal surface sorption sites are most likely blocked after humic acids adsorption, these preferential sorption sites are also located on humic acids but at lower concentration (Fig. 2). Therefore, their increased concentration on the mycelial surface facilitated Zn(II) sorption.

4 Conclusions

This paper highlights that investigation of mutual sorption interactions between humic acids and microbial surfaces is extremely important for understanding the mobility of potentially toxic metal(loid)s in the environment. While the fungal biomass constitutes a surface with high affinity for humic acids, it is highly likely that the humic acids pre-adsorbed on the microbial surfaces do not significantly contribute to their mobility because of the low sorption capacity of humic acids for As(V),

Sb(III) and Pb(II). In contrast, humic acids provide preferential sorption sites for Zn(II), enabling increased sorption capacity with increasing amount of humic acids immobilized in Ca-alginate beads. Optimum uptake here occurs at pH between 4 and 6. However, the effect of mutual interactions between humic acids and mycelial pellets on Zn(II) immobilization indicates that zinc affinity is higher for the fungal surface than it is for humic acids. These do not provide sufficient active zinc sorption sites, thus resulting in the decreased sorption capacity of mycelial pellets modified with humic acids compared to the unmodified biomass.

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