Characterization and sorption properties of *Aspergillus niger* waste biomass

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**Abstract:** The structure and the biosorption properties of fungal biomass of *Aspergillus niger* originated from citric acid fermentation industry was investigated. This waste biomass, produced in high quantity in carefully controlled industrial processes, has certain favourable characteristics that may be improved for its usefulness. In environmental chemistry, it is known for the removal of heavy metals cations. In this work, different alkaline treatments (1M NaOH/20 °C/24 h and 10M NaOH/107 °C/6 h) were used to evaluate the dependence of sorption properties of biomass on the cell wall composition. The biosorption was studied by the batch method, with the biomass concentration of 1 g/l, at pH 6. The adsorption of lead was more effective than that of cadmium. The biosorption capacity was evaluated using the biosorption isotherm derived from the equilibrium data. At pH 6, the maximum lead biosorption capacity estimated with the Langmuir model was 93 mg/g dry biomass.

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**Keywords:** fungal biomass, biosorption, chitosan, lead, cadmium

1 Introduction

*Aspergillus niger* biomass, a waste product of the industrial production of citric acid, was characterized by different analytical methods in order to evaluate the influence of a pre-treatment on the sorption properties of material.

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The biomass was characterized by elementary analysis and IR spectroscopy. IR spectra of raw and pre-treated biomass were compared to standard spectra to evaluate the effects of chemical treatment on the functional groups that may be involved in the biosorption of heavy metals.

The mycelia of various fungi, including *Phycomyces*, *Absidia*, *Aspergillus*, and *Mucor* spp., were reported by different authors to be able to remove heavy metals from aqueous solutions ([3, 7, 10]). For these applications, the use of nonliving dried biomass seems to be preferred ([4]). The exploitation of industrial waste biomass seems to be judicious because of its high quantity and uniform composition.

Structure differences and sorption properties have been compared for samples submitted to different treatment procedures. While still in the fermentation plant, mycelia were washed with dilute sodium hydroxide and, later, treated with concentrated sodium hydroxide if necessary. This treatment led to the removal of some soluble or hydrolysable components such as proteins, soluble glucans, and lipids. This step allowed for the isolation of the chitinous fraction of biomass (called also chitin-glucan complex) that plays an active role in the sorption of heavy metals ([8]). The sorption properties were investigated for lead and cadmium removal from dilute solutions.

### 2 Materials and methods

#### 2.1 Materials

*Aspergillus niger* mycelia were supplied by a fermentation plant Activa a.s. of Kaznějov, Czech Republic. The fungal culture was obtained from the deep fermentation mode of the production of citric acid. The biomass issued was abundantly rinsed by water, centrifuged, and dried in a fluidized bed drier. The sample of the raw biomass was marked sample R. The samples were then submitted to different alkaline treatments to prepare two experimental materials. The first was washed by 1M solution of NaOH at room temperature for 24 hours and then centrifuged. The second was submitted to the same treatment and then it was boiled in a 10M solution of NaOH for 6 hours and centrifuged. Finally, both samples were rinsed with water, centrifuged, and dried in a fluidized bed drier. The first sample was marked sample A, the second sample B.

Before the experiments, the samples were ground and sieved to the different size fraction: $G_1 < 125 \mu m < G_2 < 400 \mu m < G_3 < 600 \mu m$.

The solutions of heavy metals were prepared by dilution from 1000 ppm lead and cadmium standard solution (Analytika s.r.o., Praha). Sodium hydroxide was supplied by Penta, Praha.

#### 2.2 Elementary analysis and IR measurements

The content of C, H, N atoms in the samples of mycelia was determined by the Perkin-Elmer 2400 automatic elementary analyser.
The samples of mycelia and the standards (chitin, chitosan) were analysed by FTIR method using a Nicolet 740 spectrometer. The powdered and dried mycelia were mixed with KBr and conditioned to the form of disc. Before the measurement, the discs of KBr were dried for 24 hours in a vacuum in presence of silica gel. The spectra were measured with a resolution of 2 cm\(^{-1}\) using 128 accumulations of the spectrum. The programme OMNIC Software Version 4.1 was used for their manipulation.

2.3 Batch biosorption studies

A batch equilibration method was used to realise the biosorption experiments. For most experiments, 50 ml of metal solution with an initial metal concentration of 10 mg/l was used. The size of biomass particles was in the range 0.125 – 0.400 \(\mu\)m. The solutions were adjusted just before experimentation to approximately pH 6 with 1% NaOH. The mixtures of biomass and metal solution were shaken on a horizontal shaker for one hour. The time of the experiment was chosen in accordance with several authors who have found the metal uptake time in biosorption processes between 10 minutes and one hour ([1, 8]). Samples were taken out, filtered and the metal concentration was determined by an atomic absorption spectrophotometer (Varian, SpectrAA880) with flame atomisation method.

3 Results and discussion

3.1 Characterization of biomass by IR and elementary analysis

The biosorption process is dependent upon the composition of the fungal cell wall. In addition to proteins, the fungal cell wall contains glycoproteins, glucans, chitin and/or chitosan ([5]). The latter two are biopolymers, chemically similar to cellulose, that differ only by the functional group attached to carbon 2. While cellulose has a hydroxyl group in this position, chitin contains the N-acetyl group (NHCOCH\(_3\)) and chitosan contains an amine group (NH\(_2\)) (Fig. 1). The presence of the NH\(_2\) group was found to be responsible for the high chitosan adsorption capacity for metal cations. Therefore, in this work, the Aspergillus niger mycelia were treated by alkaline hydrolysis, firstly to remove the soluble components, secondly to increase the number of amino groups (this means to increase the deacetylation degree) in chitin/chitosan structure. Because the degree of deacetylation increases with increasing temperature or NaOH concentration ([2]), two different pre-treatment conditions resulting in samples A and B were chosen.

The carbon, hydrogen, and nitrogen content in the samples A and B treated by different methods were nearly the same (C: 40.2 and 39.1%; H: 7.5 and 7.4%; N: 2.3 and 2.4% respectively). For comparison, the elementary analysis of raw, non-alkali treated mycelia was made. The carbon and hydrogen content, 40.5 and 6.3%, respectively, was appropriate, but the nitrogen content of 1.9%, suggests that the alkaline treatment increased the amino groups in the material.
The IR spectra of the mycelia samples and the standards were measured in order to determine a deacetylation degree of the samples. The IR spectra of the chitin standard (Sigma-Aldrich) with the assignments of the bands is presented in the Figure 2. Figure 3 compares the IR spectra of the standards of chitin and chitosan (the second with deacetylation degree > 85%), and the samples of mycelia.

The deacetylation degree (DD) was determined according to Shigemasa ([9]). The 1560 cm$^{-1}$ band that relates to the amide vibration (amide II), was considered to be used as probe band. This choice is advantageous because the band is less affected by water in
the sample. The C-O stretching bands, 1070 and 1030 cm$^{-1}$, were considered to be used as reference bands. The ratios chosen provide a good linear relation through the range of DD from 0 to 100%. The calibration curves with the illustration of baseline methods used are illustrated in Figure 4. Already by the look of individual spectra, especially the intensities of the 1560 cm$^{-1}$ band (amide II), the DD of the samples can be estimated. It is clear that the DD of the samples is situated between the DD values of the standards. The determination of the bands ratios and the values of DD are listed in the table 1.

![Figure 3: Comparison of IR spectra of standards and samples of mycelia (sample A~SD$P_1$, sample B~SD$d$).](image)

|        | $A_{1560}$ (1900–1500) | $A_{1073}$ (1220–860) | $A_{1027}$ (1220–860) | $A_{1560}/A_{1070}$ | % DD $A_{1560}/A_{1070}$ | $A_{1560}/A_{1030}$ | % DD $A_{1560}/A_{1030}$ |
|--------|------------------------|-----------------------|-----------------------|---------------------|--------------------------|---------------------|--------------------------|
| chitin | 0,579                  | 0,737                 | 0,738                 | 0,79                | 0                        | 0,78                | 0                        |
| Sigma  |                        |                       |                       |                     |                          |                     |                          |
| chitosan | 0,069                | 0,458                 | 0,394                 | 0,15                | 87                       | 0,18                | 89                       |
| Sigma  |                        |                       |                       |                     |                          |                     |                          |
| raw mycelia | 0,199         | 0,534                 | 0,555                 | 0,37                | 58                       | 0,36                | 61                       |
| mycelia |                        |                       |                       |                     |                          |                     |                          |
| sample A | 0,042                 | 0,135                 | 0,129                 | 0,31                | 61                       | 0,33                | 67                       |
| sample B | 0,028                 | 0,164                 | 0,154                 | 0,17                | 84                       | 0,18                | 89                       |

**Table 1** Determination of the deacetylation degree (the baselines are listed in parentheses).

The average DD values of 59%, 64%, and 86% for samples R, A, and B, respectively, indicate that the sequential alkaline treatment caused an increase of deacetylation, and,
3.2 Biomass and sorbent dosage selection

Preliminary experiments were performed to determine the optimum sorbent dosage and pH, and to evaluate the efficiency of different sorbents.

The influence of sorbent dosage was tested on sample R issued directly from the production at pH 6. Biomass concentration from 1 g/l to 12 g/l was tested. It corresponds to sorbent dosage of 0.05 g to 0.60 g per 50 ml of metal solution. The results are presented in Figure 5. It is clear that the quantity of adsorbed metal in the case of Pb and Cd increase with the decreasing sorbent dosage. For subsequent experiments, the sorbent dosage of 0.05 g per 50 ml of solution was used.

Influence of pH on the adsorption capacity was tested at fixed sorbent dosage (Figure 6). Fixation of both the metals was slight at low pH. With increased pH, the adsorption capacity slightly increased. In all additional experiments, pH 6 was used in order to avoid precipitation phenomena.

The evaluation of the sorption capacity of raw and alkali-treated samples was realised under typical conditions. The quantity of adsorbed metals (concentration initial 10 mg/l) was 8.3 mg Pb and 4.5 mg Cd per g of sample R, 8.8 mg Pb and 4.7 mg Cd per g of sample A and 9.3 mg Pb and 5.2 mg Cd per g of sample B. The results indicate that the adsorption capacities of biomasses are greater for lead as compared to cadmium. In addition, the adsorption capacity increases with the extent of biosorbent pre-treatment. The mycelia sample treated in boiling sodium hydroxide achieves the highest adsorption of lead. We could expect this treatment of being able to transform weakly active sorption sites in the biomass structure to reactive functional groups. Therefore, this sample (sample B) was chosen for the measurement of lead adsorption isotherm.
Influence of sorbent dosage (sample R) on the adsorption capacity of Cd and Pb at an initial Pb and Cd concentration of 10 mg/l.

Influence of pH on the Pb and Cd biosorption at a initial Pb and Cd concentration of 10 mg/l.

3.3 Lead biosorption isotherm

The lead adsorption was tested on a fixed sorbent dosage in the solutions with different initial metal concentrations ranging from 25 to 200 mg/l. The experiment was performed on the mycelia sample B at pH 6. Figure 7a shows the results as a function of lead biosorption capacities $q$ (in mg of lead adsorbed per g of dried biomass) on the lead equilibrium concentration remaining in the solution, $C_{eq}$ (mg/l).

The experimental data correlated well with values predicted by the Langmuir equation. The linearised Langmuir isotherm (Figure 7b) can be represented as $C_{eq}/q = 1 / (q_{max} \cdot b) + C_{eq}/q_{max}$, where $q_{max}$ and $b$ are the maximum adsorption capacity and affinity, respectively. In this case the maximum adsorption capacity derived from the linear regression was 93 mg/g and constant $b = 0.0203$. Considering the presence of > 85% deacetylated chitosan in sample B, chelation on free amine groups or ion-exchange are
expected to be possible mechanisms of lead fixation. In accordance with the results, we can state that the batch biosorption method can be applied for the lead removal to a final concentration higher than 1 mg/l in order to maintain a favourable ratio sorbent/metal solution (Fig. 7c).

4 Conclusions

Fungal waste biomass from citric acid production has been analysed and the relation between the structure and biosorption properties have been investigated. Since metal biosorption from solution is predominantly due to physico/chemical interactions between the biomass and metal, morphological differences can greatly influence the biosorption process ([6]). The biomass pre-treated by 10M NaOH at 107°C for 6 hours has been found to be better metal sorbent than the raw or slightly alkali-treated samples. We can expect that such a strong treatment removed the proteins, the soluble lipids and others impurities and accessed the reactive functional groups on the biomass surface. This was demonstrated by the presence of chitosan > 85% deacetylated in this sample.
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