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Removal of Copper Ions from Dilute Solutions by *Streptomyces noursei* Mycelium. Comparison with Yeast Biomass

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ABSTRACT. Sorption properties of *Streptomyces noursei* mycelium for copper ions were compared with the accumulation competence of dried and native yeast (*Candida utilis*) biomass. The copper sorption capacity of *S. noursei* after optimization was found to be higher than that of the two other adsorbents (dried yeast biomass 82 %, native *Candida* cells 48 % of the sorption capacity of the *S. noursei* mycelium).

Artificially released metals are important pollutants of environment in ground waters and especially in industrial and agricultural treated waste waters. Copper, though it is essential to higher life, is toxic at higher concentration (*see*, *e.g*., Stadler *et al*. 2003; *ECOTOX* 2005); as a widely used material, it represents both potential and actual source of pollution.

Metals ions are usually removed from waste solutions by chemical methods: precipitation, electrodeposition and sedimentation (Babel and Kurniawan 2003). Other more sophisticated procedures such as ion exchange, use of activated carbon, or reverse osmosis, can also be used. However, all chemical, physical and electrochemical methods have their limits; they are suitable, *e.g*., for specific types of ions, and their efficiency depends on the content of organic matter in polluted solutions. These methods are frequently more effective at higher metal concentrations.

Biosorption on microbial cells as adsorbents is considered to be a cost-effective method for the removal of metals from wastes and is being developed in many research establishments. The biomass used is of various origins. Active copper and cadmium accumulation was found in a biofilm of sulfate-reducing bacteria (White and Gadd 2000). Kratochvil and Volesky (1998*a*) studied the biosorption ability of the *Sargassum* algae; they tested ion exchange of Cu, Ca and Fe ions and found that the affinity of the biomass for copper was the highest. Other authors use nonviable and/or killed biomass, from different cell types, *e.g*., actinomycetes, algae, bacteria, cyanobacteria, moulds, yeasts (Golab *et al.* 1995; Tsekova *et al.* 2000; Shakoori and Muneer 2002; Gavrilescu 2004); metal accumulation was also documented in higher fungi (Gabriel *et al*. 2001).

Biosorption includes a number of processes that reflect the complexity of biological structures: adsorption, ion exchange, precipitation, complexing, and active or passive uptake (Xu *et al.* 2004). The uptake of metals from mixtures can be predicted on the basis of ion sorption equilibrium constants determined experimentally (Kratochvil and Volesky 1998*b*) or applying mathematical models (Volesky 2003).

Like other sorptions, the biosorption of metals can be described by sorption isotherms: the best fit is usually found with the Freundlich model

$$q = k \times c^{1/b} \qquad \text{(Eq. 1)}$$

where q is an amount of the metal adsorbed on biomass (in mg/g dry mass), c is an equilibrium concentration of the metal in the solution (in mg/L) and k and b are the Freundlich constants.

The Freundlich model can be linearized and $1/b$ can be substituted by n:

$$\log q = n \times \log c + \log k \qquad \text{(Eq. 2)}$$

The constant k represents the metal bound when c = 1 mg/L; n corresponds to the intensity of adsorption over the range of the equilibrium concentrations under study.

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The influence of biomass concentration is also very important; its decrease enhances the specific metal content in the biomass but the total amount of bound metal is lower. A pH increase usually improves specific adsorption.

We compared the biosorption ability of *Streptomyces noursei* biomass with that of yeast. The actinomycete *S. noursei*, isolated from agricultural soil and classified by Ettlinger *et al.* (1958), is an industrial producer of the antifugal antibiotic nystatin. Waste mycelium from the industrial-scale production has been used for various purposes, including the biosorption of metals (Fjaervik and Zotchev 2005).

MATERIAL AND METHODS

Microorganisms, media and cultivations. Mycelium of industrial *Streptomyces noursei* strain from the production plant of *ICN–Research Institute of Antibiotics and Biotransformations*, Roztoky near Prague was obtained as a waste product of a large-scale nystatin production. Wet mycelium was washed with distilled water, dried for 2 d at 50 °C and mashed; a portion containing particles <0.16 mm was used for the biosorption. *Candida utilis* strain RIBM C8 (the collection of the *Institute of Microbiology, Academy of Sciences of the Czech Republic*, Prague) and Vitex (industrially dried *C. utilis* biomass, cultivated on Mg-bisulfite liquors and treated by a spray dryer; *Anhydro*, Denmark) were used as controls.

C. utilis was aerobically batch-cultivated in a medium composed of (g/L) D-glucose 30, yeast autolysate 7.5, peptone for bacteriology 7.5, NH_4Cl 9, K_2HPO_4 5.22, KH_2PO_4 2.75, $MgCl_2 \cdot 2H_2O$ 2, $FeSO_4 \cdot 7H_2O$ 0.002 (pH 5.4; NH_4OH control) at 34 °C. The biomass was centrifuged, washed with 10× larger volume of water and centrifuged again.

Sorption experiments were done in 500-mL Erlenmeyer flasks filled with 100 mL suspension and shaken at 2.5 Hz for 20 min (this time was determined to be satisfactory for achieving the equilibrium between the copper concentrations in the solution and bound on the sorbent; cf. Ozturk *et al*. 2004) at 25 °C. Three types of sorbents (concentrations of 0.5, 1.0, 2.5 and 5.0 g dry mass per 1 L suspension) were used. Initial concentrations of copper ions were 5, 10, 25, 50, 100 and 250 mg/L; $Cu(NO_3)_2 \cdot H_2O$ (analytical grade; *Fluka*) and deionized water were used for the preparation of solutions. pH was set to 4.5 by HNO_3 and/or NaOH and controlled during sorption runs (pH 5.0 was used as the maximum to avoid $Cu(OH)_2$ precipitation). The amount of copper bound to the sorbent was calculated from the difference between the concentration (determined by atomic absorption spectrometry using *Varian* Spectrometer AA-30) in the solution at the beginning and at the end of the experiment.

Evaluation of results. Linearized data were approximated by the least-squares regression using the Mathematica 4.1 software.

RESULTS AND DISCUSSION

Sorbent type. The highest copper accumulation was achieved with the biomass of *S. noursei* (for sorption time profiles with 2.5 g/L of sorbents *see* Figs 1 and 2). Curves of similar shapes were obtained by using other initial concentrations of sorbents; all curves had a saturation character.

Corresponding equilibrium states had the form of Freundlich isotherms (Fig. 2; calculated from Eq. *1*). The data for Vitex and *C. utilis* gave good fits; in the case of *S. noursei*, the Freundlich model does not appear not to be quite relevant. Similar results were obtained for other sorbent concentrations.

Nuhoglu *et al*. (2002) found the value 176 mg Cu/g for the green alga *Ulothrix zonata* under conditions similar to the maximum theoretical adsorption capacity given by the Langmuir model. Mattuschka and Straube (1993) determined for *S. noursei* the equilibrium value q = 9.0 mg Cu/g (at initial Cu concentration 63.6 mg/L, biomass concentration 3.53 g/L, temperature 30 °C, pH 5.5). We found q = 19.8 mg/g with initial concentration of Cu 50 mg/L and biomass concentration 2.5 g/L; however, when an appropriate curve (Fig. 1) was used for finding the value corresponding to the initial Cu concentration 63.6 mg/L (under conditions of Mattuschka and Straube 1993) our q value was 26.5 mg/g. Employing the data for biomass concentration of 5.0 g/L and assuming a roughly linear relation between the adsorptions for biomass concentrations 2.5 and 5.0 g/L (cf. Fig. 3) we can conclude that our q value estimated according to Mattuschka and Straube (1993), reached ≈21.0 mg/g, *i.e*. twice higher than that of Mattuschka and Straube (1993).

The highest amount of copper adsorbed per 1 g of biomass (k; *see* Table I) was found for *S. noursei*, the lowest with *C. utilis*. Moreover, *S. noursei* showed an inverse dependence of k on sorbent concentration (with decreasing sorbent concentration the k value increased). The constants correspond to those of Sag *et al.*

(2000) who found similar values of n and k in the same range for *Rhizopus arrhizus*. We found a higher adsorption capacity in *S. noursei* and Vitex that in *R. arrhizus*.

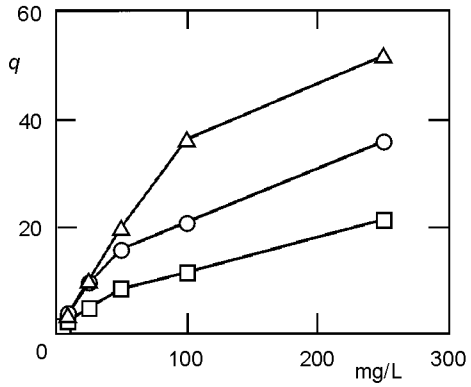

Fig. 1. Dependence of the adsorption capacity (q, mg/L) on the initial concentration of copper (c_0, mg/L) at pH 4.5 (concentration of sorbents 2.5 g/L); *triangles* – *S. noursei*, *circles* – Vitex, *squares* – *C. utilis*.

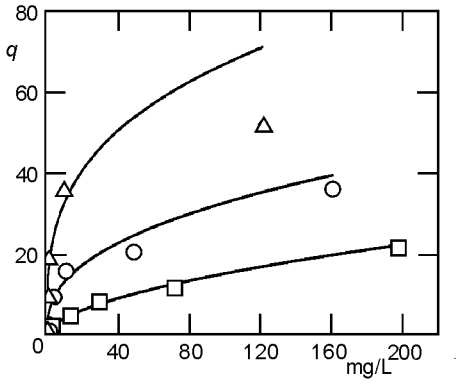

Fig. 2. Adsorption isotherms (q, mg/L) of copper (c_0, mg/L) approximation measured data by the least-squares regression using the Mathematica 4.1 software; for further details *see* Fig. 1.

The high adsorption ability of the *S. noursei* mycelium for copper is also demonstrated by the linearized adsorption isotherms in Fig. 3. Similar results were also obtained by Ozturk *et al*. (2004) with *Streptomyces coelicolor*; their data for Ni^{II} and Cu^{II} adsorption excellently fitted with the Freundlich isotherm.

Table I. Constants[a] of the linearized Freundlich isotherms at pH 4.5

Sorbent	Concentration[b]	n	k	log k
S. noursei	0.5	0.254	27.991	1.447
Vitex		0.386	8.281	0.918
C. utilis		0.568	2.542	0.405
S. noursei	1.0	0.278	17.314	1.238
Vitex		0.298	9.733	0.988
C. utilis		0.602	1.361	0.134
S. noursei	2.5	0.309	16.003	1.204
Vitex		0.386	5.443	0.736
C. utilis		0.547	1.192	0.076
S. noursei	5.0	0.464	9.176	0.963
Vitex		0.305	6.459	0.810
C. utilis		0.502	1.538	0.187

[a]Constants of linearized Freundlich isotherms (Eq. *2*) obtained by approximation for all examined concentrations of the sorbents; the approximation was done by the method of least squares.
[b]g/L dry mass.

Effect of pH. The highest adsorption capacity was achieved at pH 5 (Table II). pH enhanced the q of Vitex and *C. utilis*. At pH 5.0 we reached a mere 82 % (Vitex) and 48 % (*C. utilis*) of the adsorption capacity of *S. noursei*. Sag *et al*. (2000) found the best adsorption of copper for *R. arrhizus* at pH 4 while at pH 5 it was significantly lower. This can be connected with a different composition of biomass surfaces at various pH. On the other hand, Ruiz-Manriquez *et al.* (1998) achieved with *Thiobacillus ferrooxidans* a higher adsorption capacity at pH 6 (the copper perchlorate solution they used started to precipitate at pH 6.5).

On comparing the results of Ozturk *et al*. (2004), who found q = 42 mg/g in *S. coelicolor* at initial copper concentration of 150 mg/L and biomass concentration of 1.0 g/L (our estimation was 55.2 mg/g), and Mattuschka and Straube (1993) at 63.6 mg/L and 3.5 g/L, who found q = 9 mg/g for *S. noursei* (our estimation was 19.5 mg/g), we demonstrated a higher adsorption ability of *S. noursei* relative to *S. coelicolor* (by

30 %) and also higher than found earlier for *S. noursei* (by 100 %). We also showed that the adsorption ability of *S. noursei* is higher than that of the two types of yeast biomass.

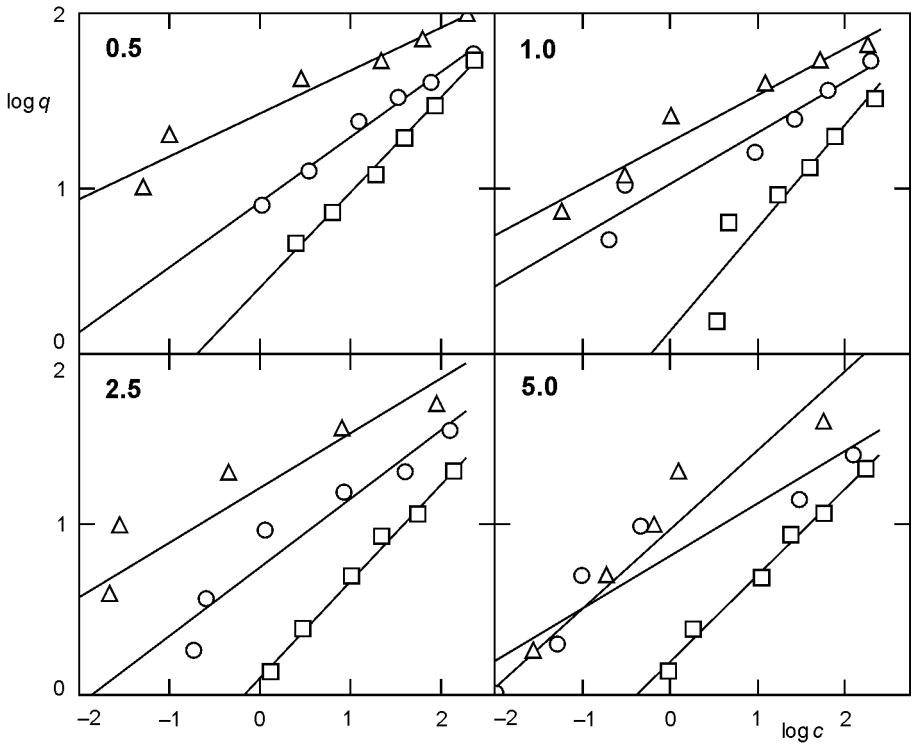

Fig. 3. Linearized adsorption isotherms ($\log q$, mg/g) of copper ($\log c$, mg/g) approximating measured data (constants *n* and *k* were calculated from Eqs *1* and *2* to simplify the approximation); 0.5–5.0 – concentration of sorbents in g/L; for further details *see* Figs 1 and 2.

Table II. Effect of pH (3.0–5.0) on adsorption capacity[a]

Sorbent	3.0	4.0	4.5	5.0
S. noursei	8.44	6.17	11.4	18.5
Vitex	17.5	20.7	22.8	31.7
C. utilis	33.1	36.7	36.1	38.6

[a]Sorbent concentration 2.5 g/L, initial Cu concentration 100 mg/L.

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REFERENCES

BABEL S., KURNIAWAN T.A.: Low-cost adsorbents for heavy metals uptake from contaminated water: a review. *J.Hazard.Mat.* **97**, 219–243 (2003).

ECOTOX (*ECOTOXicology*) *Database 2005*: Toxicity studies for copper chloride (anhydrous) on all organism groups – toxicology studies from the primary scientific literature on aquatic organisms. US Environmental Protection Agency, Washington (DC) 2005; **http://www.epa.gov/ecotox/**.

ETTLINGER L., CORBAZ R., HUTTER R.: Zur Systematik der Actinomyceten. 4. Eine Arteinteilung der Gattung *Streptomyces*. *Arch. Mikrobiol.* **31**, 326–358 (1958).

FJAERVIK E., ZOTCHEV S.B.: Biosynthesis of the polyene macrolide antibiotic nystatin in *Streptomyces noursei*. *Appl.Microbiol.Biotechnol.* **67**, 436–443 (2005).

GABRIEL J., BALDRIAN P., HLADÍKOVÁ K., HÁKOVÁ M.: Copper sorption by native and modified pellets of wood-rotting basidiomycetes. *Lett.Appl.Microbiol.* **32**, 194–198 (2001).

GAVRILESCU M.: Removal of heavy metals from the environment by biosorption. *Eng.Life Sci.* **4**, 219–232 (2004).

GOLAB Z., BREITENBACH M., JEZIERSKI A.: Sites of copper-binding in *Streptomyces pilosus*. *Water Air Soil Pollut.* **82**, 713–721 (1995).

KRATOCHVIL D., VOLESKY B.: Biosorption of Cu from ferruginous wastewater by algal biomass. *Water Res*. **32**, 2760–2768 (1998*a*).
KRATOCHVIL D., VOLESKY B.: Advances on the biosorption of heavy metals. *Tibtech* **16**, 291–300 (1998*b*).
MATTUSCHKA B., STRAUBE G.: Biosorption of metals by a waste biomass. *J.Chem.Technol.Biotechnol.* **58**, 57–63 (1993).
NUHOGLU Y., MALKOC E., GÜRSES A., CANPOLAT N.: The removal of Cu(II) from aqueous solutions by *Ulothrix zonata*. *Biores.Technol*. **85**, 331–333 (2002).
OZTURK A., ARTAN T., AYAR A.: Biosorption of nickel(II) and copper(II) ions from aqueous solution by *Streptomyces coelicolor* A3(2). *Colloids Surf.B-Biointerfaces* **34**, 105–111 (2004).
RUIZ-MANRIQUEZ A., MAGANA P.I., LOPEZ V., GUZMAN R.: Biosorption of Cu by *Thiobacillus ferrooxidans*. *Bioproc.Eng*. **18**, 113–118 (1998).
SAG Y., KAYA A., KUTSAL T.: Lead, copper and zinc biosorption form bicomponent systems modelled by empirical Freundlich isotherm. *Appl.Microbiol.Biotechnol*. **53**, 338–341 (2000).
SHAKOORI A.R., MUNEER B.: Copper-resistant bacteria from industrial effluents and their role in remediation of heavy metals in wastewater. *Folia Microbiol*. **47**, 43–50 (2002).
STADLER N., VÁCHOVÁ L., KRASOWSKA A., HÖFER M., SIGLER K.: Role of strategic cysteine residues in oxidative damage to the yeast plasma membrane H^+-ATPase caused by Fe- and Cu-containing Fenton reagents. *Folia Microbiol*. **48**, 589–596 (2003).
TSEKOVA K.V., MARINOV P.G., TZEKOVA A.N.: Copper accumulation by *Aspergillus awamori*. *Folia Microbiol*. **45**, 217–220 (2000).
VOLESKY B.: Biosorption process simulation tools. *Hydrometallurgy* **71**, 179–190 (2003).
WHITE C., GADD G.M.: Copper accumulation by sulfate-reducing bacterial biofilms. *FEMS Microbiol.Lett*. **183**, 313–318 (2000).
XU H., TAY J.H., FOO S.K., YANG S.F., LIU Y.: Removal of dissolved copper(II) and zinc(II) by aerobic granular sludge. *Water Sci. Technol*. **50**, 155–160 (2004).