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Use of the Industrial Yeast *Candida utilis* for Cadmium Sorption

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ABSTRACT. The sorption ability of *Candida utilis* biomass for cadmium ions with accumulating competence of dried cells and cells in alginate was compared. After an optimization of process conditions (pH 5.5, biomass concentration 1 g/L and c_0 50 mg/L), the cadmium sorption capacity of dried yeast biomass was perceptibly higher than that of the other tested adsorbents. Considering the sorption of the dried yeast biomass equal to 100 %, the cells in alginate reached 86 % while native cells showed only 42 %.

The removal of cations of 'heavy' metals from industrial waste waters or recovery of metals from their solutions as part of their mining by leaching can be accomplished by biotechnological methods that make use of microorganisms as cation sorbents (Babel and Kurniawan 2003). Microbial biomass derived from, *e.g.*, actinomycetes (Selatnia *et al*. 2004), cyanobacteria, other bacteria, algae (Lodeiro *et al*. 2005; Akhtar *et al*. 2003), moulds and yeasts (Salinas *et al.* 2000; Baldrian 2003), especially *C. utilis* (Fencl *et al*. 1974) has the ability to accumulate metal ions and radionuclides from the environment.

The amount of accumulated cations can be very large and depends on many factors ranging from the microbial species and its physiological state to external physico-chemical conditions such as pH (Bagy *et al*. 1991), temperature, *etc*. (Xu *et al*. 2004). Other important factors affecting the accumulation capacity are the type of the cation, its valency, the presence of other cations or anions, complexing agents, inhibitors of microbial growth, resistance to stress factors (Olasupo *et al*. 1993), *etc*.

The mechanisms of metal binding to microbial biomass can be roughly divided into three main types (Kujan *et al.* 2005): intracellular accumulation (this process requires live cells), sorption or complex formation on cell surface (it takes place on both live and dead cells) and extracellular accumulation or precipitation (the process may require viable cells). The metabolically independent biosorption of metals by yeast cells occurs within several minutes (Kratochvil and Volesky 1998; Volesky 2003).

Biomass concentration has a marked effect on sorption. With increasing pH and decreasing biomass concentration the metal concentration in the biomass tends to increase. However, using lower biomass concentrations, the absolute amount of bound metal also decreases. Process optimization requires the use of a suitable biomass concentration at a suitable pH value, with concomitant meeting of other prerequisites, *e.g*., economical (Volesky 1994).

Cadmium contributes markedly to environmental pollution, especially of waters and soil. It is usually not found in high concentrations but the toxicity of cadmium ions is high relative to that of other 'heavy' metals. These low concentrations can conveniently be removed by low-cost, low-energy biosorption, which is, at the same time, a highly efficient process.

This study compares the sorption ability of three types of sorbents derived from the industrial yeast *C. utilis* for cadmium cations at various pH values and biomass concentrations.

MATERIAL AND METHODS

Microorganisms, media and cultivation conditions. Candida utilis strain RIBM C8 (deposited in the collection of the *Institute of Microbiology, Acad. Sci. Czech Rep*., Prague) was cultivated in a medium containing (g/L): D-glucose 30, yeast autolysate 7.5, peptone for bacteriology 7.5, $MgCl_2{\cdot}2H_2O$ 2; K_2HPO_4 5.22; KH_2PO_4 2.75; NH_4Cl 9; $FeSO_4{\cdot}7H_2O$ 0.002. Batch aerobic cultivation took place at pH 5.4 (NH_4OH control) and temperature 30 °C. The biomass was centrifuged, washed with a 10× larger volume of water and centrifuged again.

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Sorption methods. Immobilized biomass was obtained by alginate immobilization of native biomass; the amount of bound yeast cells was determined from the nitrogen balance of native cells *vs.* the immobilized cell granules. Metal samples were prepared by using $Cd(NO_3)_2{\cdot}4H_2O$ (analytical grade; *Fluka*). Sorption experiments were done in 500-mL Erlenmeyer flasks with 100 mL solutions on a reciprocal shaker (160 Hz, 1 h). The required pH of 4.5 was set at the beginning of the experiment and adjusted in the course of the adsorption with 0.1 mol/L HNO_3 and/or 0.1 mol/L NaOH. The use of the three sorbents based on *C. utilis* was examined at biomass concentrations of 1.0, 2.5 and 5.0 g/L, initial concentrations of the cadmium ions were 5, 10, 20, 30, 50, 75, and 100 mg/L at a constant temperature of 25 °C and at pH 3.5, 5.4, and 6.5. The amount of adsorbed metal was determined from the difference between metal concentrations in the solution before and after sorption. The metal concentration was determined by atomic absorption spectrometry (*Varian* Spectrometer AA-30).

Evaluation of results. Data were approximated using the Mathematica 5.2 software (package Statistics-Nonlinearfit).

RESULTS AND DISCUSSION

The highest absorbed amounts referred to the cell dry mass in the polymer were reached with dried yeast biomass (Fig. 1 *left*). The plot of the amount of absorbed metal (q) against its equilibrium concentration in the solution (c) fits the characteristics of an isotherm. The data measured with 1 g/L of sorbent were

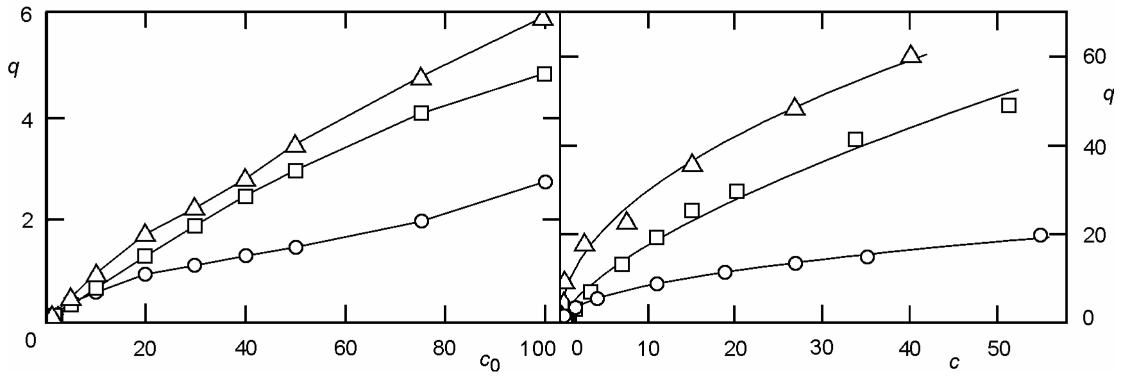

Fig. 1. Efect of initial (c_0; *left*) and equilibrium (c; *right*) Cd^{2+} concentration (in mg/L) on the adsorbed amount of cadmium (q; mg/g dry yeast mass); pH 5.4, sorbent concentration 1 g/L; *triangles* – dried yeast biomass, *squares* – cells in alginate, *circles* – native cells.

fitted with Freundlich isotherms (Fig. 1 *right*; for computation method *see* Kujan *et al.* 2005). The highest absorbtion was achieved with dried biomass, followed by cells immobilized in alginate, with relatively large scatter of values around the isotherm. Freundlich isotherms were also constructed for various combinations of sorbent concentrations and pH values; the isotherm constants calculated for initial Cd^{2+} concentrations of 75–100 mg/L and the most widely used pH 5.4 are given in Table I. At higher concentrations, the experimental curves lose the character of isotherms. Dried cells displayed the highest specific adsorption, followed

Table I. Constants n and K of Freundlich isotherms at pH 5.4 for cadmium adsorption

Sorbent	g/L dry mass	n	K
Dried cells	1.0	0.49	9.62
	2.5	0.38	8.97
	5.0	0.46	8.80
Cells in alginate	1.0	0.66	3.81
	2.5	0.64	3.18
	5.0	0.80	2.11
Native cells	1.0	0.48	2.81
	2.5	0.48	2.23
	5.0	0.46	1.61

by alginate-immobilized cells. The accumulation capacity of native *C. utilis* cells was by far the lowest; this points to the importance of a suitable pretreatment of the sorbent (whose economical requirements should, however, not exceed the advantages gained by the higher adsorption ability).

Of greater relevance for practical use is the fraction of cadmium removed from the original solution. Using native cells and the lowest sorbent concentration (Fig. 2 *left*) the best results were achieved at pH 5.4 while with increasing cadmium concentration the adsorption increased predominantly at pH 6.5. The pH 6.5 was the most suitable at a sorbent concentration of 5 g/L which was the highest from the viewpoint of the absolute adsorbed amount. At this concentration the removal of cadmium was ≥80 % over the whole range of initial concentrations (Fig. 2 *right*) (therefore, at this concentration the capacity of the sorbent was not fully utilized).

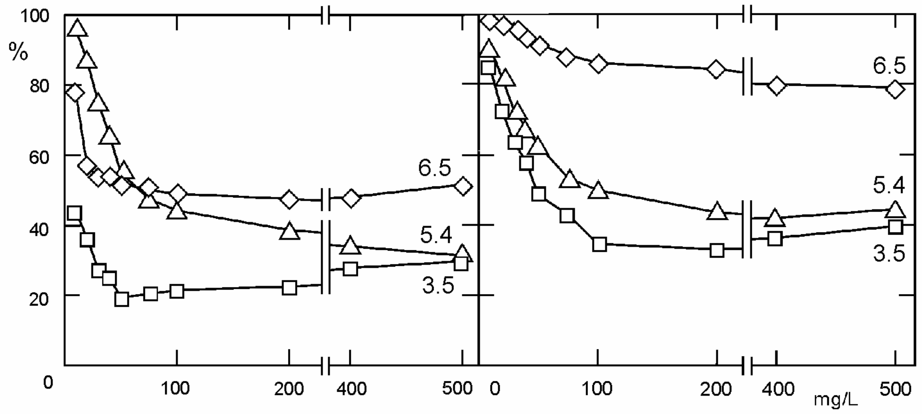

Fig. 2. Percentage of Cd^{2+} removal from the solution as affected by the initial metal concentration (0–500 mg/L) and pH (3.5, 5.4 and 6.5); concentration of native biomass 1 (*left*) and 5 g/L (*right*).

We compared our results with data of other authors: Salinas *et al.* (2000) in their study of biosorption properties of *Rhodotorula rubra* isolated from mine waters reached with viable biomass q_{10} = 4.05 mg/g dry mass (at 10 mg/L Cd equilibrium concentration) and for nonviable biomass 9.8 mg/g (both at pH 4.0 and 25 °C). Volesky *et al.* (1993) reported 3 mg/g dry mass for viable cells of *Saccharomyces cerevisiae*. We reached for viable cells at pH 3.5 a value of q_{10} = 5.5 mg/g dry mass, at pH 5.4 the same parameter was 8.7 mg/g. When nonviable biomass was used, q_{10} = 28 mg/g dry mass was found.

White-rot (wood-rotting) fungus *Pleurotus ostreatus* was able to accumulate cadmium at the amount of 20 mg/g dry mass (Favero *et al.* 1991; Baldrian 2003). Lodeiro *et al.* (2005) with various brown seaweeds calculated for cadmium q_{max} = 60–70 mg/g dry mass from a pseudo-second order sorption rate model and experimental data at pH ≥ 4.5 and higher. When they used for evaluation the Langmuir isotherm, the values of q_{10} varied from 24 to 38 mg/g, which is comparable with our results with *C. utilis*.

Our study showed that sorbents based on *C. utilis* biomass can conveniently be used for cadmium biosorption from aqueous solutions. After optimization, the results point to more advantageous parameters than with the biomass from other yeast species and are comparable with other, often hardly available and expensive, biosorption materials.

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