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Biosorption of copper by yeasts

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Summary. The ability to accumulate copper from aqueous solutions was determined with different yeast species. Yeast cells did not show any significant differences in process kinetics. The uptake was very fast and was influenced by environmental factors. The metal-accumulating capacity differed among the tested strains. The yeasts *Candida tropicalis* and *Pichia guilliermondii* were chosen for extensive research. Cells of the stationary growth phase were able to adsorb a high amount of copper. The uptake capacity decreased with increasing biomass concentration. Copper adsorption obeyed the Freundlich isotherm. Optimal pH range was between 5 and 7. The biomass could be used repeatedly for biosorption after desorption by mineral acids.

Key words: Bioaccumulation – Biosorption – Metal uptake – Copper – Yeasts

Introduction

The increasing pollution of the environment with metalliferous wastes demands the development of new methods for removal and recovery of metals from sewage sludge and industrial effluents. Recently, microbial biomass has been considered for use as an adsorbing agent for removal of heavy metals from aqueous systems. Certain species of microorganisms are able to accumulate large amounts of heavy metals from their external environment. *Pseudomonas aeruginosa* cells can concentrate uranium up to about 50% of their own dry mass and in *Zoogloea ramigera* up to 40% of cell mass was found to be copper (Strandberg et al. 1981; Norberg and Rydin 1984). Accumulation of metals by microbial cells can be a metabolic or nonmetabolic process. Nonmetabolic accumulation may be a result of binding of metals to cell-surface components, of reduction of metals, of precipitation by formation of hydroxides or sulphides, and intracellularly of diffusion. Metabolism-dependent intracellular accumulation involves specific ion transport systems.

For the practical use of microorganisms to recover or to separate metals from aqueous systems, the amount of metals which can be accumulated by microbial cells is very important. A large number of strains were screened for their ability to take up amounts of copper. In the literature, data concerning accumulation of copper by yeasts vary (Kikuchi 1964; Baldry and Dean 1980; Gadd and Mowll 1985). Because of the different experimental conditions, the results obtained are not comparable. This paper describes the adsorption of copper by different yeast strains under identical conditions. The influence of cell age, biomass density, contact time, initial copper concentration and external pH on accumulation capability has been studied.

Materials and methods

Microorganisms and cultivation. Yeast strains used in this study were derived from culture collections of the University of Greifswald [*Candida catenulata* SBUG 515, *Candida mesenterica* SBUG 519, *Candida parapsilosis* CBS 604, *Candida rugosa* SBUG 60, *Candida sake* CBS 2920, *Cryptococcus terreus* SBUG 278, *Lodderomyces elongisporus* CBS 2605, *Sporodiobolus ruinenii* SBUG 722, *Trichosporon cutaneum (beigelii)* SBUG 27], of the Institute of Biotechnology in Leipzig (*Candida utilis* H 92, *Yarrowia lipolytica*) and of the Technical University of Merseburg (*Candida famata* CBS 18, *Candida tropicalis* HP 15, *Debaryomyces hansenii* VKM Y 102, *Pichia guilliermondii* H4, *Rhodotorula* sp. M2, *Sacharomyces cerevisiae*). Cells for copper uptake experiments were cultivated in 100 ml of a complex nutrient broth with 10 g/l glucose in 500-ml culture flasks at 30° C on a rotary shaker for 24 h. Yeast cells were harvested by centrifugation, washed twice and resuspended in distilled water.

Uptake experiments. Experiments were carried out in 500-ml culture flasks with volumes of 100 ml on a rotary shaker at 30° C. Copper sulphate ($CuSO_4 \cdot 5H_2O$) was used for adsorption in different concentrations. 5 mM morpholinoethane sulfonic acid (MES) served as buffer. The suspension was adjusted to pH = 5.5 with 100 mM tetramethylammonium hydroxide solution. At this pH all copper is in the ionic form. After the adsorption process,

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the cells were collected by centrifugation and metal concentration in the supernatant was measured by atomic adsorption spectrometry (AAS). The concentration of copper in the biomass was calculated from the difference of metal concentration before and after the adsorption process. As a control, the biomass was treated with 1 M HCl to solubilize the bound copper and then also measured by AAS.

Estimation of dry mass. Cell mass concentration was adjusted by measuring the absorbance of cell suspensions at 600 nm and comparing it to a calibration curve obtained by determination of cell dry mass.

Results and discussion

Copper accumulation by different yeasts

For the 17 different yeast strains screened under discontinuous conditions with various biomass concentrations and initial concentrations of copper, the amounts of copper adsorbed by the cells differed considerably. The results are summarized in Table 1.

Velocity of copper uptake

The high velocity of metal uptake (see Fig. 1) is a great advantage for practical purposes and indicates an adsorption process. A high concentration of metal ions was rapidly bound to the cell surface. In all cases, equilibrium was reached after an hour. Therefore, all further experiments were completed after 1 h.

Experiments with *Candida tropicalis* on copper uptake in the presence of glucose and phenol led only to insignificantly better results than those without any nutrients. Generally, metabolism-independent uptake is fast and reversible. The surface of yeast cells can act as an ion exchanger with reversible binding of cations. Phosphoryl and carboxyl groups are important in metal complexation (Volesky 1988).

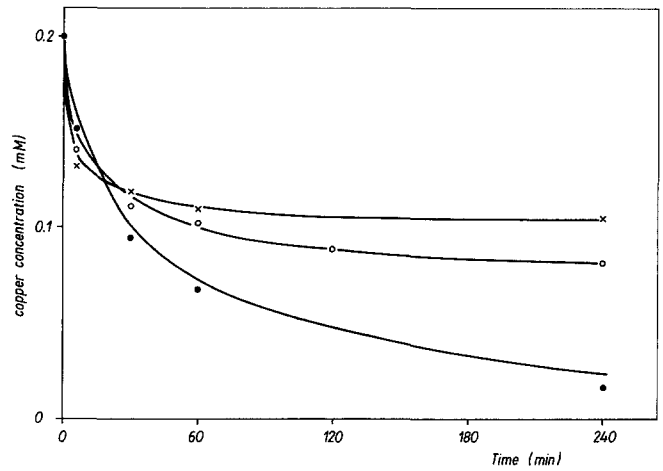

Fig. 1. time course of copper uptake by *Candida tropicalis.* pH = 5.5, temperature = 30° C, initial copper concentration in solution = 0.2 mM. Dry mass: (×) 1.4 g l^{-1}; (○) 3.1 g l^{-1}; (●) 6.1 g l^{-1}

Influence of cell age

Figure 2 shows the dependence of copper accumulation on cell age. Cells at the stationary growth phase had the highest uptake capacity. Therefore, all experiments were carried out with 24-h-old cultures. Studies by McMurrough and Rose (1967) had shown that phosphorus and protein contents of yeast cell walls increased with decreasing growth rate. This may be a reason for more metal binding sites.

Table 1. Uptake of copper by different yeast strains at pH 5.5, 30° C, with contact time of 1 h

Yeast strain	Accumulated Cu (mg g^{-1}) [cf. initial Cu (%)] with dry mass			
	3 g l^{-1}, [Cu]=		1 g l^{-1}, [Cu]=	
	0.2 mM	2 mM	0.2 mM	2 mM
Candida catenulata	2.0 [48]	16.9 [40]	4.1 [32]	35.8 [28]
C. famata	3.0 [71]	18.4 [43]	7.1 [55]	35.7 [28]
C. mesenterica	1.9 [46]	16.8 [40]	6.8 [58]	43.4 [38]
C. parapsilosis	2.4 [57]	19.8 [47]	6.5 [51]	41.1 [32]
C. rugosa	1.7 [40]	18.6 [44]	3.2 [32]	39.2 [31]
C. sake	1.7 [37]	25.2 [55]	4.3 [31]	55.8 [41]
C. tropicalis	1.9 [44]	23.1 [55]	4.7 [37]	60.9 [48]
C. utilis	2.2 [51]	16.2 [38]	6.1 [48]	34.1 [27]
Cryptococcus terreus	1.1 [25]	23.7 [56]	4.2 [33]	71.8 [56]
Debaryomyces hansenii	2.7 [62]	27.9 [55]	6.8 [55]	58.1 [38]
Lodderomyces elongisporus	2.2 [52]	20.5 [48]	4.4 [35]	55.3 [43]
Pichia guilliermondii	2.9 [65]	16.6 [38]	7.3 [55]	34.0 [26]
Rhodotorula sp.	3.0 [71]	16.8 [40]	6.8 [54]	49.1 [40]
Saccharomyces cerevisiae	2.7 [61]	20.7 [46]	6.8 [51]	43.3 [32]
Sporodiobolus ruinenii	1.3 [29]	23.7 [55]	7.6 [59]	70.4 [55]
Trichosporon cutaneum	2.3 [54]	20.1 [48]	6.2 [49]	66.8 [53]
Yarrowia lipolytica	2.9 [69]	24.0 [57]	6.9 [54]	38.4 [30]

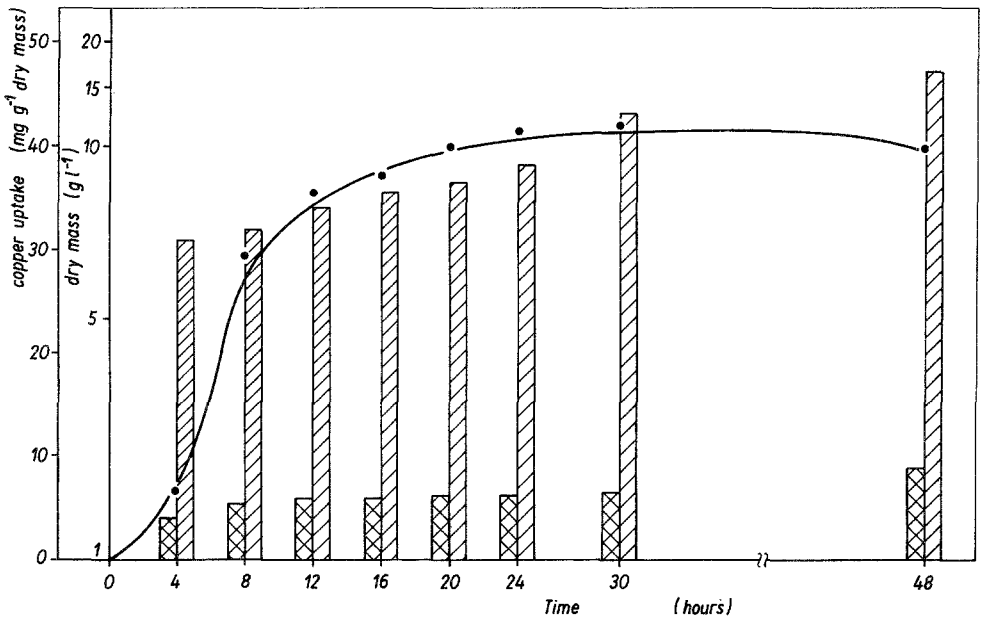

Fig. 2. Effect of cell age on copper uptake by *Candida tropicalis*. pH = 5.5, temperature = 30° C, contacting time 1 h. Initial copper concentration in solution: (□) 0.2 mM; (□) 2 mM; (●) dry mass

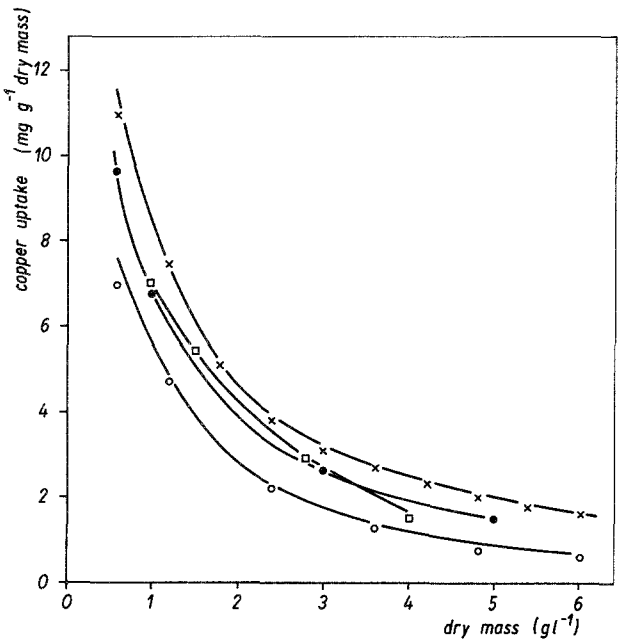

Fig. 3. Copper uptake by different yeast cells. pH = 5.5, temperature = 30° C, contacting time 1 h; initial copper concentration in solution 0.2 mM. (×) *Pichia guilliermondii*; (○) *Candida tropicalis*; (□) *Rhodotorula* sp.; (●) *Saccharomyces cerevisiae*

Both living and dead cells are able to accumulate copper. The use of dead biomass eliminates the toxicity problems. Cells of *C. tropicalis* and *Pichia guilliermondii* were killed by boiling for 30 min. In contrast to other authors who observed increased metal uptake by dead cells of various microorganisms (Horikoshi et al. 1981; Nakajima et al. 1981), the copper uptake by living yeast cells was only slightly higher than by dead cells.

Influence of cell density

The quantity of accumulated copper varied among the species, but all yeasts showed a dependence on cell concentration as shown in Fig. 3 for some strains.

The biomass concentration was found to have a significant effect on adsorption. The uptake capacity decreases with increasing cell density. On the other hand, it is possible to obtain a better removal of metals from solution with a higher density of biomass. This agrees with results concerning the uptake of copper by different filamentous fungi (Horikoshi et al. 1981; de Rome and Gadd 1987). Itoh et al. (1975) showed with different metals that *Saccharomyces cerevisiae* cells adsorb more metal at low cell concentrations than at higher ones. Reasons for this dependence could be electrostatic interactions, interference between binding sites or reduced mixing at high biomass concentrations.

Influence of copper concentration

The copper concentration influenced the uptake strongly, as shown in Fig. 4. The copper accumulated in the biomass increased with increasing initial concentration of copper. The Freundlich equation can be applied to describe the relationship between the amount of copper adsorbed/biomass and the remaining copper concentration in solution at equilibrium. Both *C. tropicalis* and *P. guilliermondii* obey the Freundlich isotherm. The intended model describes a monolayer adsorption. Similar results were also obtained with other organisms and metals (Kuyucak and Volesky 1989; Sag and Kutsal 1989; Röhricht et al. 1990).

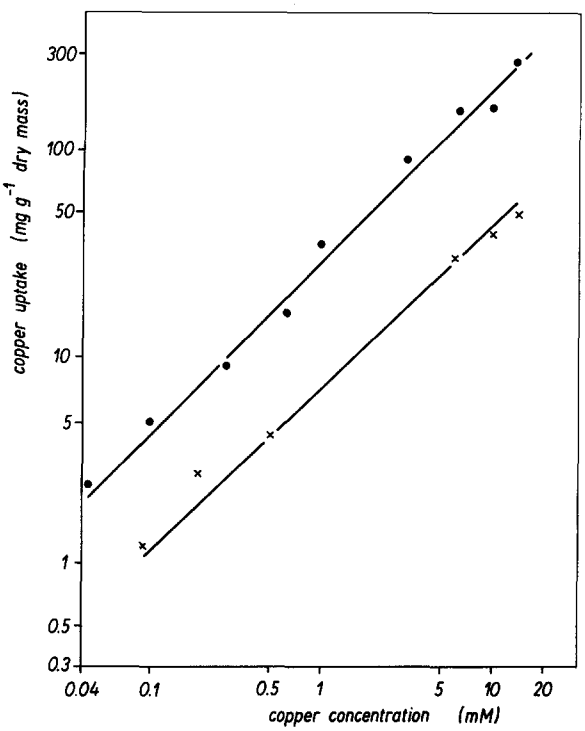

Fig. 4. Effect of initial copper concentration in solution on uptake by cells of *Pichia guilliermondii*. pH = 5.5, temperature = 30° C, contacting time 1 h; dry mass: (●) 0.5 g l^{-1}; (×) 5 g l^{-1}

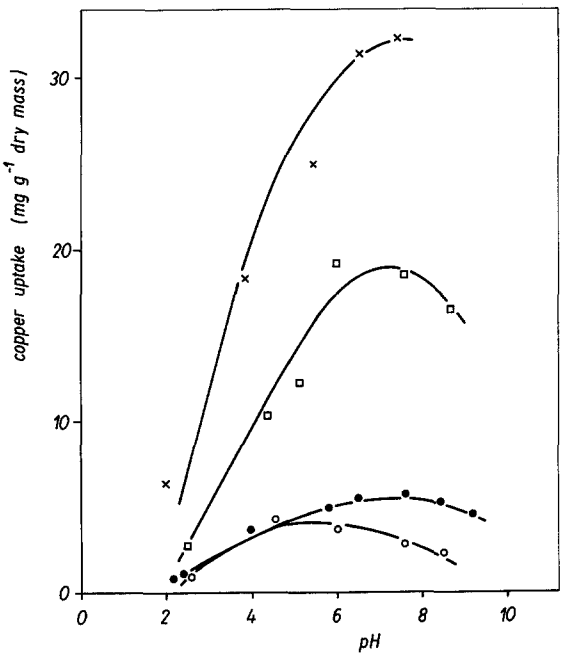

Fig. 5. Influence of pH in solution on copper uptake by *Candida tropicalis* and *Pichia guilliermondii*. pH = 5.5, temperature = 30° C, dry mass of the cells 3 g l^{-1}; initial copper concentration in solution: *C. tropicalis* (●) 0.4 mM, (×) 1.6 mM; *P. guilliermondii* (○) 0.4 mM, (□) 1.6 mM

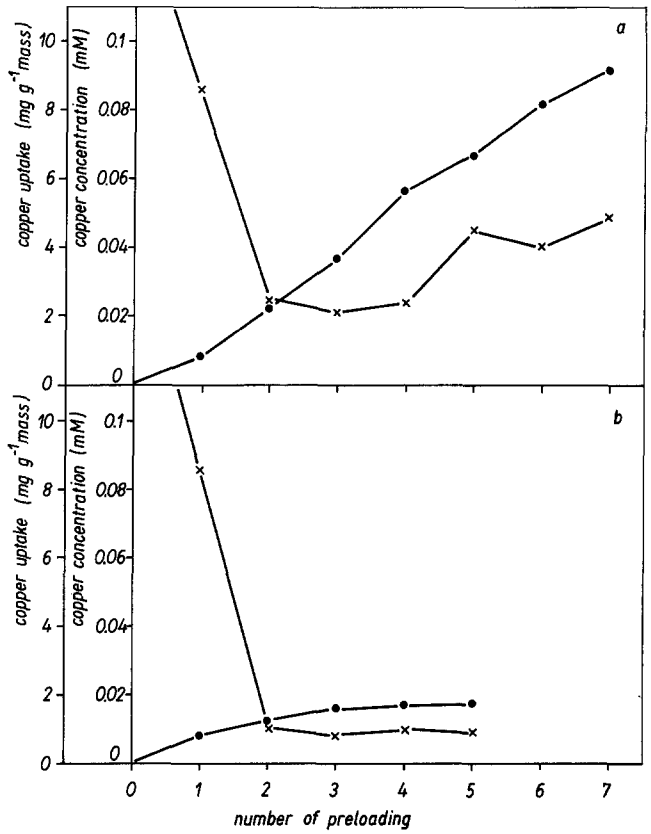

Fig. 6. Preloading of *Candida tropicalis* cells with copper sulphate solution containing 0.16 mM copper. pH = 5.5, temperature = 30° C, dry mass of the cells 6 g l^{-1}; desorption with 1 M HCl: (a) without desorption; (b) with desorption; (×) copper rest concentration; (●) copper uptake

Influence of pH

The optimal pH for biosorption of copper was between 5–8 (Fig. 5). Other authors have also described the dependence of adsorption of metals on pH and found pH 5–7 to be effective (Wang and Wood 1984; Kuyucak and Volesky 1988a). The pH of the solution affects the activity of binding groups on the cell surface. At pH values of 4–5, copper sulphate is ionized and a large number of cell-surface carboxylate groups are dissociated. Interactions between the positively charged metal ions and the negative functional groups of the biomass cause the biosorption (Kuyucak and Volesky 1988b).

Desorption and resorption

Technical applications of microbial metal accumulation demand regeneration of the biomass for reuse in a multi-step arrangement. The desorbing material should be highly efficient, cheap and should not modify the biomass. Cells of *C. tropicalis* (6 g l^{-1}) were incubated repeatedly with an initial copper concentration of 0.16 mM. For desorption, the cells were treated with 1 M HCl. The results, with and without intermittant desorptional steps, are shown in Fig. 6. After every load-

ing step the cells were still able to accumulate copper, but the adsorption capacity of these cells decreased and the copper concentration remaining in solution increased. On the other hand, with desorption after each adsorption, a constant copper concentration after each step was obtained. These results are in agreement with those obtained for uranium uptake by *Streptomyces* (Nakajima and Sakaguchi 1986).

The treatment of *C. tropicalis* cells with diluted acids (10% acetic acid, 10% lactic acid, 50% nitric acid or 1 M hydrochloric acid) could remove copper completely. Similar results has been described by Strandberg et al. (1981) and de Rome and Gadd (1987). It is also possible to desorb the metal with organic complexing agents (Dunn and Bull 1983). In our experiments with *C. tropicalis* 0.1 M EDTA could remove the initial copper by only 60%.

The results described here should serve as a basis for further studies towards an application of accumulating processes using waste biomass.

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