Communications to the Editor

Cadmium Biosorption by Saccharomyces cerevisiae

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Cadmium uptake by nonliving and resting cells of *Saccharomyces cerevisiae* obtained from aerobic or anaerobic cultures from pure cadmium-bearing solutions was examined. The highest cadmium uptake exceeding 70 mg Cd/g was observed with aerobic baker's yeast biomass from the exponential growth phase. Nearly linear sorption isotherms featured by higher sorbing resting cells together with metal deposits localized exclusively in vacuoles indicate the possibility of a different metal-sequestering mechanism when compared to dry nonliving yeasts which did not usually accumulate more than 20 mg Cd/g. The uptake of cadmium was relatively fast, 75% of the sorption completed in less than 5 min.
Key words: biosorption • biosorbent • *Saccharomyces cerevisiae* • cadmium biosorption • metal uptake • brewer's yeasts • baker's yeasts

INTRODUCTION

Cadmium, well recognized for its negative effect on the environment where it accumulates throughout the food chain, is being used in a wide variety of industrial processes, e.g., alloy preparation, metal plating, and electronics. Passive[3,7,16] as well as active[5,11–13] or enzymatically driven[6] microbial uptake of cadmium has been well documented. Various species of yeasts were shown to sequester cadmium from solution. While *Saccharomyces uvarium* and *Candida utilis* accumulated 0.13 mmol Cd/g cells,[10] *Saccharomyces cerevisiae* accumulated 0.16 mmol Cd/g cells via an energy dependent process.[9] Preliminary screening of different biomass types for their affinity toward sequestering cadmium revealed some differences and an interesting potential associated with the biomass of a common yeast *S. cerevisiae.*[8]

MATERIALS AND METHODS

Commercially distributed "active" dried and wet pressed biomass of *Saccharomyces cerevisiae* (strains 1452-L6F+8% 1453-L65 as baker's yeast) was supplied by Lallemand, a yeast company in Montreal, Canada. Brewer's yeast (wet pressed and dried biomass of *S. cerevisiae*) was obtained from Molson Breweries, Montreal, Canada. The strains of baker's and brewer's yeasts supplied were subsequently cultivated in the laboratory on the medium containing 10 g glucose, 5 g peptone, 3 g malt extract, and 3 g yeast extract in 1 L of distilled water. Following cultivation in 100-mL Erlenmeyer flasks on a rotary shaker (25°C, 24 h, 2.5 Hz), the biomass harvested from the exponential growth phase (at approximately 15 h) was washed with distilled water. These "resting cells" were then used for the equilibrium and kinetics sorption experiments (approximately 0.2 g of live biomass).

Chemicals, instrumental analysis [atomic absorption spectrometry (AAS)], sorption contact, and calculation procedures (q) were used as reported earlier.[2]

Electron micrographs were made on a Philips transmission electron microscope (model 410) at an accelerating voltage of 80 kV using a 40-μm aperture. Following centrifugation (7000 rpm for 15 min), glutaraldehyde fixation (3% solution in a 0.1 phosphate buffer), and alcohol washing, the cells were embedded in Spurr-epoxy resin at 70°C overnight. Because of interest in experimental heavy metal deposition, the samples were not treated with metal solutions for electron microscopy. Sections (800 Å thick) were cut with a Sorvall MT2B Microtome and the presence of the metal in the cell confirmed by using an EDAX probe analysis (JEOL JEM-100CX electron microscope with an EDAX J-100 C-154-10 detection unit and a 707-A EDAX unit).

RESULTS AND DISCUSSION

Cadmium biosorption isotherms for *S. cerevisiae* are summarized in Figure 1. Baker's yeast generally demonstrated slightly higher cadmium uptake than brewer's yeast. This difference was observed not only in the resting-cell biomass (from the exponential growth phase) but also in the commercial wet pressed or dried biomass. Only the wet pressed cells of brewer's yeast attained full cadmium saturation in the experiments. The calculation of q_{max} values using the Langmuir sorption model was attempted to assist in the comparison of biosorption performance, particularly in cases where the sorbent biomass materials did not reach their full saturation in experiments. Different physiological states of the yeast biomass (fresh from the exponential growth phase or commercial wet pressed older cells) also resulted in profound differences in the cadmium uptake. Highly metabolically active cells from the exponential growth phase likely contain highly active enzymes, some of which may be active in complexing and binding the

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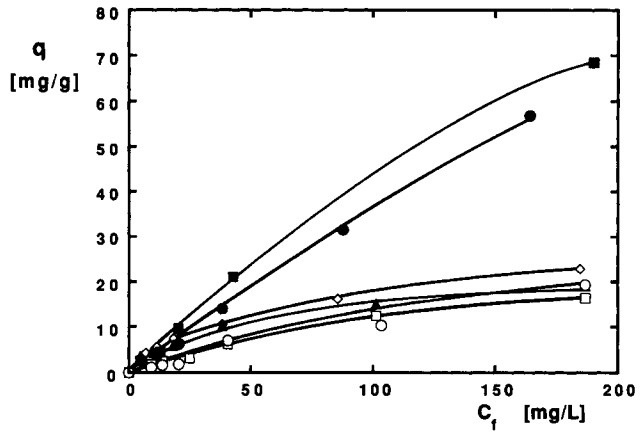


Figure 1. Cadmium sorption isotherms at pH 4.5 for *S. cerevisiae:* (■) baker's yeast resting cells from the exponential growth phase; (●) brewer's yeast resting cells from the exponential growth phase; (▲) commercial wet pressed baker's yeast biomass; (□) commercial wet pressed brewer's yeast biomass; (◇) baker's yeast, dry commercial biomass; (○) brewer's yeast, dry commercial biomass.

metal.[6] They could also have enough energy reserves for active transport of cadmium metal eventually deposited into the vacuoles. This mode of metabolically mediated metal uptake is not based on sorption and has been designated as bioaccumulation. It can be expected that the classical sorption Langmuir model will not be able to reasonably well predict bioaccumulation behavior, and, correspondingly, the predicted q_{max} values cannot reflect the reality (Table I). The active transport of cadmium into the cell resulted in a linearly increasing isotherm while the Langmuir model predicts exponential metal uptake. The final concentration value of 200 mg Cd/L was chosen as a higher end residual concentration at which the metal uptakes q_{200} are compared. The comparison of theoretical and experimental values are summarized in Table I.

A Langmuir sorption model was used to evaluate the sorption behavior of the materials examined. It served to estimate the maximum metal uptake values where they could not be reached in the experiments. Its constant b can serve as an indicator of the isotherm rise in the region of lower residual concentrations which reflects the "strength" or "affinity" of the sorbent material for the solute. The higher the value of b, the desirably steeper the isotherm. The model, which is based on assumptions of surface adsorption,[4] showed a good fit where (passive bio-) sorption prevailed.[2] However, in cases of a more complex metal uptake apparently involving more complex, perhaps metabolically driven bioaccumulation and deposition of the metal (resting-cell biomass originating from the exponential growth phase or the "active dry yeast"),[8] the model did not fit the experimental data and its predictions were unrealistic.

Figure 2 shows that the rate of cadmium uptake examined for resting yeast cells originating from the exponential growth phase was very fast. Within the first 3 min of

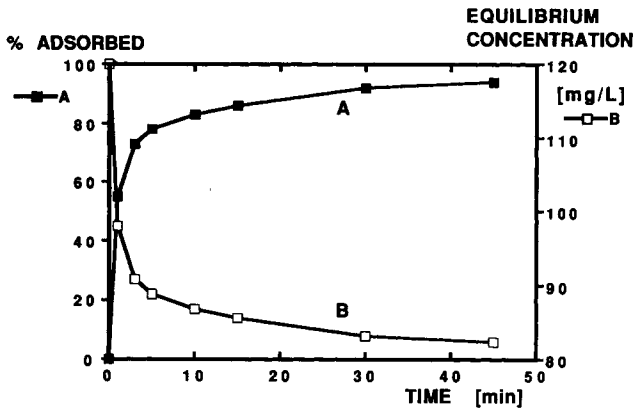


Figure 2. Time profile of cadmium sorption by resting cells of *S. cerevisiae* (brewer's yeast).

Table I. Experimental and calculated cadmium uptake by different types of sorbent materials.

Sorbent Type	Experimental[a]		Langmuir Parameters[a]				Difference[b]		
	q_{10} (mg/g)	q_{200} (mg/g)	q_{10} (mg/g)	q_{200} (mg/g)	q_{max} (mg/g)	b ($\times 10^2$)	q_{10} (%)	q_{200} (%)	R^{2f}
Baker's *S. cerevisiae*									
growing	4	71	3	59	(1586)[c]	0.02	33	10	NS[d]
wet pressed	5	20	4	19	22	2.56	25	5	0.98
dry	5	23	5	23	28	1.98	0	2	0.98
Brewer's *S. cerevisiae*									
growing	4	66	4	64	(1171)[c]	0.03	0	10	NS[d]
wet pressed	2	17	2	17	32	0.59	0	0	0.88
dry	1	20	1	20	38	0.39	0	0	NS[d]
Duolite GT-73	28	63	26	62	67	6.43	8	2	0.97
Amberlite (RA-400)	2	29	3	27	56	0.46	−33	6	0.67[e]

[a] q_{10} and q_{200} metal uptake at the residual concentrations of 10 and 200 mg/L, respectively.
[b] $(q_{EXP} - q_{CAL})\ 100/q_{CAL}$
[c] Values of Langmuir q_{max} in brackets are unrealistically high because the sorption process involved in these cases may not be a passive biosorption but rather active bioaccumulation where the Langmuir model fails to fit the experimental data.
[d] Nonsignificant.
[e] Significant at $P < 0.05$.
[f] Significant at $P < 0.01$.

contact, 73% of the metal uptake was completed. Electron micrographs of cadmium-exposed resting yeast cells from the exponential growth phase in Figure 3 indicated that the electron-dense metal deposits were predominantly accumulated within large vacuoles. The deposits became enlarged with the increasing time of exposure to the cadmium-containing solution. The cadmium uptake of the biomass examined was 58 mg Cd/g cells. The cell wall could not be distinguished due to omitted contrasting treatment. There was no apparent alteration of the cell interior and organelles of the cadmium-exposed cells. This is in contrast to the yeast cells exposed for the same prolonged period of time to uranium.[8] Those were devoid of vacuoles, and their organelles could not be distinguished. These comparative observations may serve as an indication of different metal deposition mechanisms at work.

The presence of cadmium deposits in the vacuoles was confirmed by means of EDAX spectra (Fig. 4). For the native cells the energy level of the cadmium spectral *M*-line of the EDAX spectrum remained at background levels. The same was true when the probe was focused on the inner cell wall region or the cytoplasmic region even of the cadmium-containing cells. It was only the electron-dense areas in the vacuoles which contained the cadmium deposits. The conspicuous phosphorus peak on the EDAX spectrum of cadmium-bearing cells indicates that the metal may be deposited in the form of cadmium phosphate which may be the result of phosphatase activity in the cell.[6] An interesting fact in these experiments was that there were no cadmium phosphate deposits on the outer cell wall of the yeast cells and that some yeast cells exhibited no cadmium deposition at all. This again would indicate a metabolically driven cadmium deposition. Similar observations in conjunction with uranium and *Streptomyces* were also made earlier.[15]

The common yeast *S. cerevisiae* was demonstrated to be a reasonably potent biosorbent material for cadmium. Aerobically propagated yeast biomass (baker's yeast) was always a better sorbent that anaerobically propagated brewer's yeast. Due to the fact that cadmium was observed to

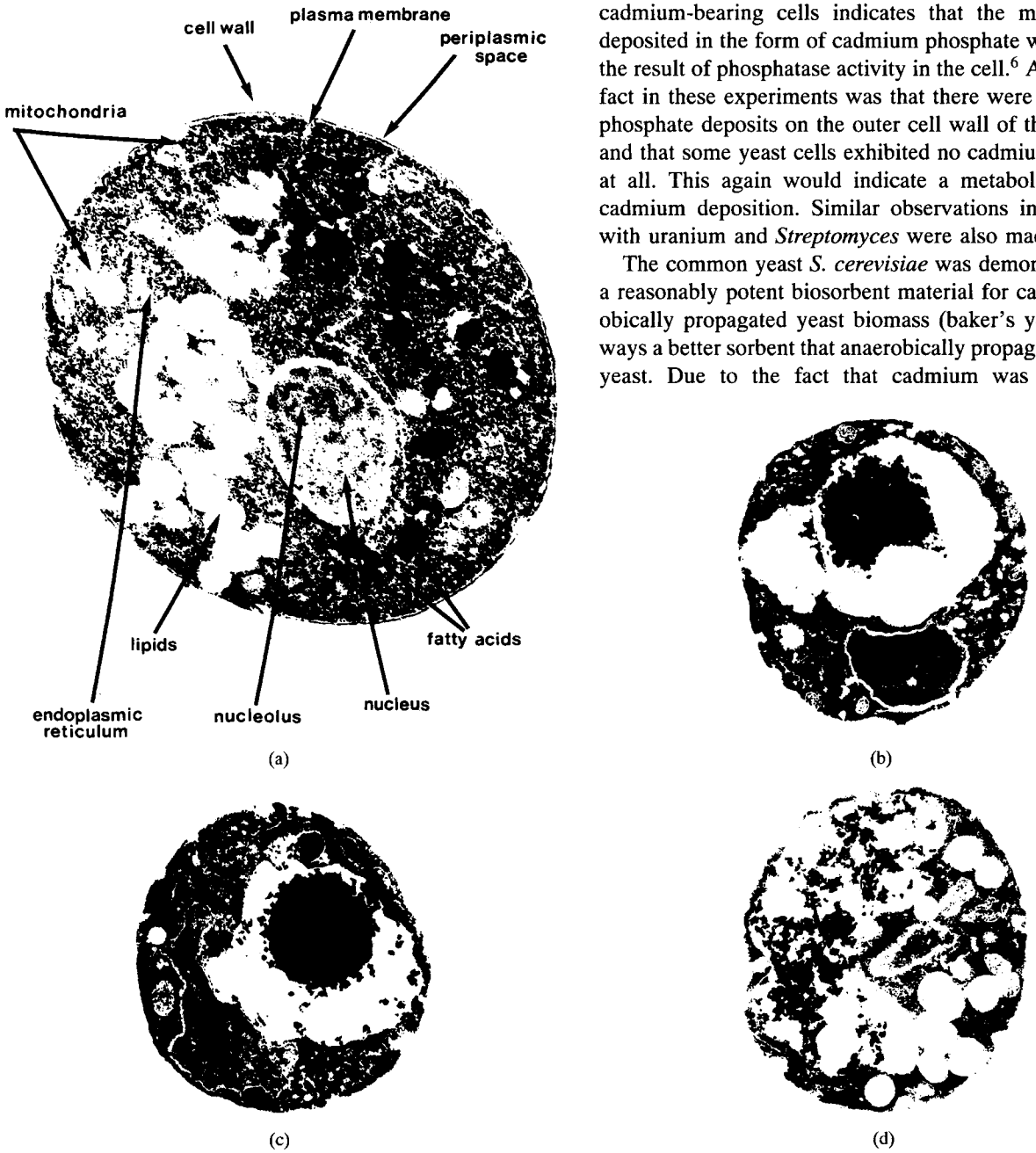
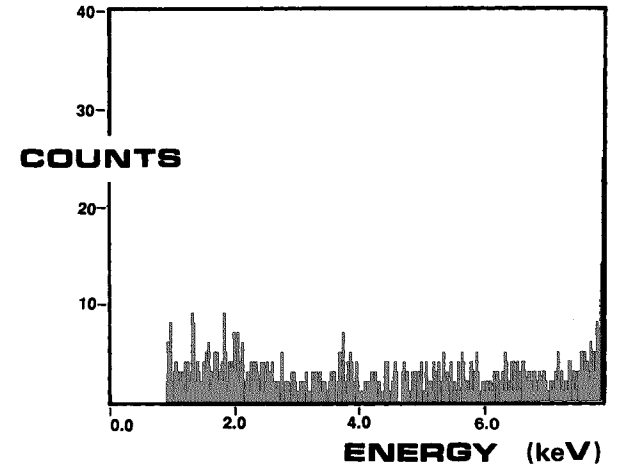
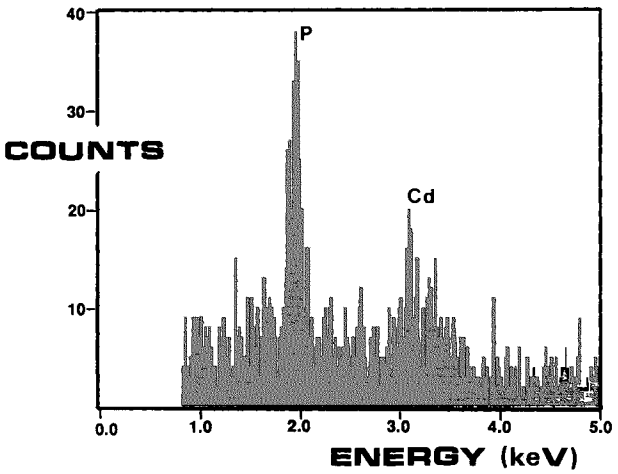


Figure 3. Electron micrographs (27,000×) of *S. cerevisiae:* yeast cells (resting cells from exponential phase at approximately 15 hours): (a) a native cell (36,000×, original magnification); Cd contact: (b) 5 minutes, (c) 5 hours, (d) 15 hours.



(a)



(b)

Figure 4. A typical x-ray EDA spectrum for *S. cerevisiae* yeast cells: (a) cadmium spectral M-line for native cells, for inner cell wall and cytoplasm; (b) cadmium spectral M-line and phosphorus line for vacuoles of cells exposed to cadmium-containing solution (pH 4.5).

form insoluble phosphate deposits in vacuoles of living cells and not on the surface of the cell wall where the metal sorption on the layers of phosphomannan could be expected, another metal deposition mechanism can be suspected based on the active transport of ions. Early on, it was observed that cadmium affects the permeability of the yeast cell membrane causing a loss of K^+ and Mg^{2+} ions from the cell.[14] *Saccharomyces cerevisiae* has been reported to preferentially accumulate Cd^{2+} and other divalent cations by energy dependent metabolism.[9] Two K^+ ions were released for each divalent cation transported inside after activation of the cell surface receptors and opening of the divalent channels in the plasma membrane originally existing for Ca^{2+} cations. The uptake of divalent cations is competitive and pH dependent,[9] and in the case of Ca^{2+}, its deposition in polyphosphate complexes has been reported.[1] The accumulation of the metal in the polyphosphate deposits resulted from entering of Cd cations into the $Ca^{2+}/n\text{-}H^+$ antiport system which is driven by a proton-motive force generated by the vacuolar membrane H^+–adenosinetriphosphatase system.[1] The cell vacuoles are known to serve as a collection site for surplus ions and molecules. It may be of interest to study the cadmium uptake and accumulation by the yeast cells in more detail, which was considered beyond the scope of this work.

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