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ORIGINAL PAPER

B. Volesky · H. A. May-Phillips

# Biosorption of heavy metals by *Saccharomyces cerevisiae*

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**Abstract** Abundant and common yeast biomass has been examined for its capacity to sequester heavy metals from dilute aqueous solutions. Live and non-living biomass of *Saccharomyces cerevisiae* differs in the uptake of uranium, zinc and copper at the optimum pH 4–5. Culture growth conditions can influence the biosorbent metal uptake capacity which normally was: living and non-living brewer's yeast: U > Zn > Cd > Cu; non-living baker's yeast: Zn > (Cd) > U > Cu; living baker's yeast: Zn > Cu ≈ (Cd) > U. Non-living brewer's yeast biomass accumulated 0.58 mmol U/g. The best biosorbent of zinc was non-living baker's yeast ( ≈ 0.56 mmol Zn/g). Dead cells of *S. cerevisiae* removed approximately 40% more uranium or zinc than the corresponding live cultures. Biosorption of uranium by *S. cerevisiae* was a rapid process reaching 60% of the final uptake value within the first 15 min of contact. Its deposition differing from that of other heavy metals more associated with the cell wall, uranium was deposited as fine needle-like crystals both on the inside and outside of the *S. cerevisiae* cells.

## Introduction

Biosorption of metals has been recently receiving a great deal of attention for both its scientific novelty and application potential. It is a property of dead biomass, particularly of microbial origin, to retain and concentrate metallic elements from relatively dilute solutions. Of special interest are "industrial" heavy metals for their toxicity in the environment or metals of technlogical interest. The types of microbial biomass of interest can quite pragmatically be those that can be easily obtained in larger quantities. In fact, industrial fermentation activities are often accompanied by significant disposal problems with regard to the used-up microbial biomass.

One of the most ubiquitous biomass types utilized on a large scale by man for centuries is yeasts. Strains of *Saccharomyces cerevisiae*, when propagated aerobically, have been known as "baker's yeast", other strains of the same species, when used anaerobically and producing ethanol have been labelled "distiller's" or "brewer's yeast". Earlier observations noting the capacity of these microbial strains to sequester heavy metals have been largely scattered and inconsistent, focusing mainly on the nutritional requirements or toxicology of the yeast. Copper has for centuries been a metal in contact with the microbial yeast culture. Among others, Norris and Kelly (1979) studied the extent and selectivity of metal uptake by various live species of yeasts. Copper accumulated at a relatively high level in *Rhodotorula mucilaginosa* (0.225 mmol Cu/g cells) and even *Candida utilis* (0.18 mmol Cu/g cells) whereas the three species of *Saccharomyces* sequestered copper in the same range as *S. cerevisiae* (0.163 mmol Cu/g cells).

Various species of yeasts also have the ability to sequester cadmium from solution. Cadmium, being one of the "big three" toxic metals, is of particular interest. *S. uvarium* and *C. utilis* were reported (Norris and Kelly 1979) to accumulate approximately 0.13 mmol Cd/g. *S. lipolytica* and *R. mucilaginosa* cadmium accumulation was similar to that observed in some bacteria. *S. cerevisiae* has recently been examined for cadmium uptake, accumulating 0.16 mmol Cd/g (Volesky et al. 1993), which, however, was lower than observed for a fungus *Rhizopus arrhizus*, for example (Tobin et al. 1984). Living cells of *S. cerevisiae* were reported (Norris and Kelly 1977) to accumulate $Cd^{2+}$ via an energy-dependent process, although it is not known whether $Zn^{2+}$ could be substituted in this transport system even though *S. cerevisiae* forms similar complexes with these cations. The amounts of zinc accumulated by

B. Volesky (✉) · H. A. May-Phillips
Department of Chemical Engineering, McGill University, 3480 University St., Montreal, Qué., Canada H3A 2A7

*S. cerevisiae* were much smaller (0.004 mmol Zn/g) when compared (Mowll and Gadd 1983) to those sequestered by *Sporobolomyces roseus* (0.029 mmol Zn/g). The live-yeast accumulation of zinc was biphasic, involving rapid, metabolism-independent binding to the cell surfaces, followed by a slower phase of metabolism-dependent intracellular uptake. It was suggested that the differences in the surface metal-binding capacities were due to differences in the cell wall structure. The cell walls of *S. cerevisiae* are composed of glucan and mannan with traces of chitin whereas the walls of *Sporobolomyces roseus* contain chitin and mannan with traces of glucan and 4-aminobutyric acid. *S. cerevisiae* also showed a tendency to accumulate $Pb^{2+}$ at very similar levels to $Co^{2+}$ and this was apparently not an energy-mediated reaction, pointing more towards true "biosorption" as opposed to metabolism-based "bioaccumulation". An exhaustive and relatively up-to-date summary of the biosorbent behavior of different types of inactive microbial biomass is presented in a recent review (Volesky and Holan 1994), while a book by Volesky offers a broader discussion of biosorption (Volesky 1990a).

The problem with individual studies of metal biosorption in the literature is that most of them have been carried out for quite other purposes than examination of biosorption, and represent a very inconsistent collection. Typical are the observations on the biosorption of uranium by *S. cerevisiae* and *Pseudomonas aeruginosa* initiated on the basis of unrelated waste-water denitrification studies (Shumate et al. 1978, 1980; Strandberg et al. 1981). A relatively large amount of sequestered uranium (0.52 mmol U/g from $UO_2^{2+}$ solution) was observed, surprisingly, accumulated by only 32% of the *S. cerevisiae* cells exposed. While the culture growth conditions seemed to have some effect on the uranium uptake, the possibility of a very rapid rate of uranium uptake by the biomass was not fully appreciated by the investigators until they focused on the difficult examination of biosorption uptake kinetics (Tsezos 1980; Tsezos and Volesky 1981). More recent work with yeasts (Brady and Duncan 1993; Mattuschka et al. 1993) focuses on passive biosorption but does not satisfy the quantitative aspects.

Since *S. cerevisiae* is such a common microorganism and its biosorbent behavior in conjunction with heavy metals, while intriguing, has not been consistently assessed, this effort aims to contribute to the knowledge in this area, supplementing the earlier study (Volesky et al. 1993) which focused only on cadmium biosorption by *S. cerevisiae*.

## Materials and methods

A standard strain of *S. cerevisiae* was obtained from the McGill University Culture Collection (Department of Microbiology). A strain of *S. cerevisiae* 1452-L6F + 8% 1453-L65 (baker's active dry yeast) was obtained from Lallemand Inc., a yeast-producing company in Montreal, Canada. Another strain of *S. cerevisiae* (brewer's yeast) was supplied by Molson Breweries (Montreal, Canada). Fisher Dry Yeast (by Standard Brands Inc.) was purchased directly in a food store. While industrial fresh wet and washed biomass for experiments was obtained directly from the above local companies, it was dried when required. The strains were also aerobically subcultured in 500-ml conical flasks with 150 ml sterilized simple liquid growth medium containing (g/l): glucose (10), peptone (5), malt extract (3), yeast extract (3). Rotary-shaker incubation at 180 rpm and 25°C was normally terminated in the late exponential phase of culture growth unless specified otherwise. The specific growth rates of the subcultivated cultures were: McGill culture 0.693 $h^{-1}$, baker's yeast 0.288 $h^{-1}$, and the brewer's yeast 0.231 $h^{-1}$.

Aliquots of wet cells, containing 0.2 g dry cells, at selected phases of growth were obtained through centrifugation (Sorvall RC-5 Superspeed centrifuge at 10 000 rpm, 15 min). All cell samples were washed with distilled de-ionized water before being contacted with the metal-bearing solution(s). The cell mass, obtained by the same procedure followed by overnight oven drying at 80°C, was considered as dead biomass without further quantitative cell-survival tests.

The batch equilibrium metal-sorption studies used 0.2-g samples of dried and ground (pestle and mortar) biomass or a suitable aliquot of live biomass in 500-ml conical flasks containing 200 ml metal-bearing solution with known initial metal concentration. Nitrate salts of metals were used in the preparation of the solutions. Ample contact time was allowed for the sorption system to reach equilibrium, normally 16 h on a rotary shaker at 180 rpm and 25°C. Preliminary tests with all yeast strains indicated a good metal sorption uptake between pH 4.0 and pH 5.0, the lower value was adopted for further studies. The pH was maintained at 4 by addition of small amounts of either 0.1 M $HNO_3$ or potassium biphthalate solution when and as required, while the dilution of the original metal-bearing solution was considered negligible. All the pure chemicals were of analytical grade supplied by Fisher Scientific (Montreal).

Exposed biomass was removed by filtration through a 0.45-μm-pore-size cellulose acetate filter membrane (Millipore) and the supernatant analyzed for residual (equilibrium) metal concentation using a Perkin-Elmer (model 403) atomic absorption spectrophotometer. The uranium concentration was determined colorimetrically (uranyl-arsenazo complex; Tsezos and Volesky 1981) on a Spectronic 70 spectrophotometer (Bausch and Lomb). The detection limits of these methods are below 1 mg/l.

Uptake of metal ions ($q$) was calculated from a metal mass balance yielding (Volesky 1990b): $q$ (mmol metal/g dry biomass) $= V(C_i - C_f)/m\,M$ where $V$ is the sample volume (l), $C_i$ and $C_f$ are the initial and field metal concentrations (mg/l) respectively, $m$ is the amount of dry biomass (g) and $M$ is the relative molecular mass of the metal. Experimental control samples with no biomass added were treated identically as blanks, all adjusted to the same pH 4.0 as the sorption samples.

Electron micrographs were prepared 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 M phosphate buffer, pH 4.0), and ethanol washing, the cells were embedded in Spurr epoxy resin at 70°C overnight. Because experimental heavy metal deposition was being investigated, the samples were not treated with metal solutions for electron microscopy. Sections (800 nm thick) were cut with a Sorvall MT2B microtome and the presence of the metal in the cell confirmed by using an energy-dispersive X-ray analysis (JEOL JEM-100CX electron microscope with EDAX J-100 C-154-10, 707-A EDAX unit).

## Results

### Effect of solution buffering on metal uptake

During the process of biosorption there may be a spontaneous pH change in the solution which affects the

metal uptake. With live washed cells used in biosorption tests it was observed that the older they are the larger the pH change of the metal contact system. The most conspicuous pH increase of 1.3 pH units was observed for 24-h old biomass contacted for a 20-h period. A pH increase of only 0.3 pH unit resulted from a 24-h contact period of younger biomass (9 h). The necessary pH control or buffering of the solution can affect the sorption. The pH control additives or the buffer have to be carefully selected so that they will not interfere with the biosorbent metal uptake.

The effect of the potassium biphthalate buffer on the equilibrium biosorption of selected metals by *S. cerevisiae* biomass (strain 1452-L6F + 8% 1453-L65 from Lallemand Inc., Montreal) was examined in a batch equilibrium sorption system at a starting pH of 4.0. While the uptake of strongly sorbed uranyl ion was very slightly depressed in the presence of buffer (Fig. 1a), the buffer did not work effectively since the pH of the sorption system still changed somewhat (from pH 4.0 to 4.2). Similar but much more pronounced trends with regard to the depression of metal biosorption by the presence of buffer were also observed for copper and zinc as seen in Fig. 1b, c respectively. Addition of potassium biphthalate lowered the uptake capacity of the biomass and did not significantly contribute to the control of the pH drift. Consequently, it was decided to control pH in all equilibrium sorption experiments by adding miniscule amounts of 0.1 M $HNO_3$ rather than to use buffer.

## Uptake of metal ions by dead *S. cerevisiae* biomass

Uranium biosorption results for three different species of dry *S. cerevisiae* are summarized by isotherms in Fig. 2a. While dry brewer's yeast (Molson Brewery, Montreal) featured the highest uptake capacity of 0.592 mmol U/g cells, baker's active dry yeast (Lallemand Inc., Montreal) sequestered only low amounts of uranium (0.20 mmol U/g cells).

By contrast, Lallemand active dry yeast exhibited a higher uptake of zinc (0.47 mmol Zn/g cells at an equilibrium concentration of 2.6 mmol Zn) than other baker's (Fisher) or anaerobic brewer's (Molson) yeast strains (Fig. 2b). However, while full sorption saturation was always achieved at given residual concentrations of the metal (equilibrium), the maximum saturation metal uptake was not achieved in any of the three biomass types becasue of experimental difficulties with controlling the pH and occasional metal precipitation at elevated equilibrium metal concentrations causing irregularities in the quantitative assessment of the sorption phenomenon.

The maximum copper uptake, which would be beyond the practical biosorption application range, however, was also not even attained for the Fisher baker's yeast strain, which demonstrated a considerably higher

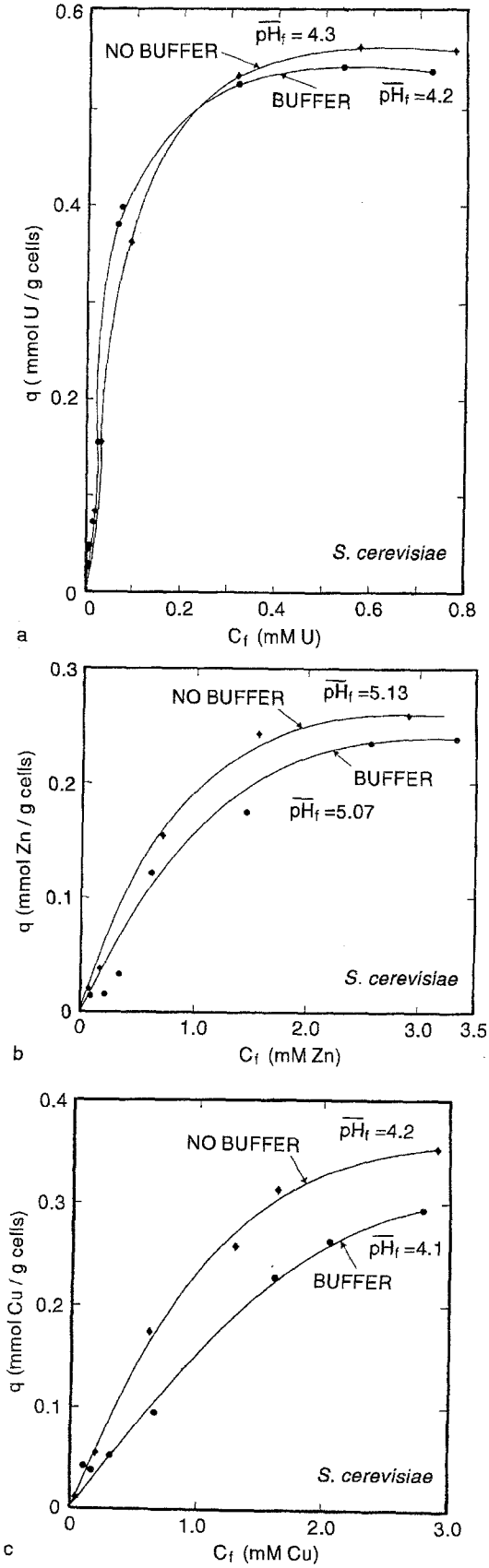
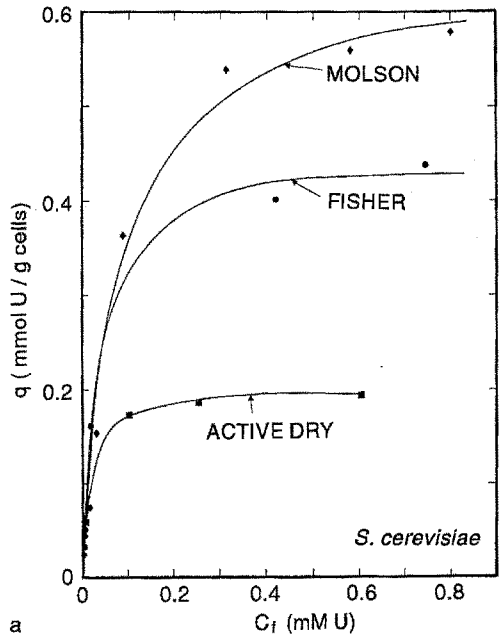
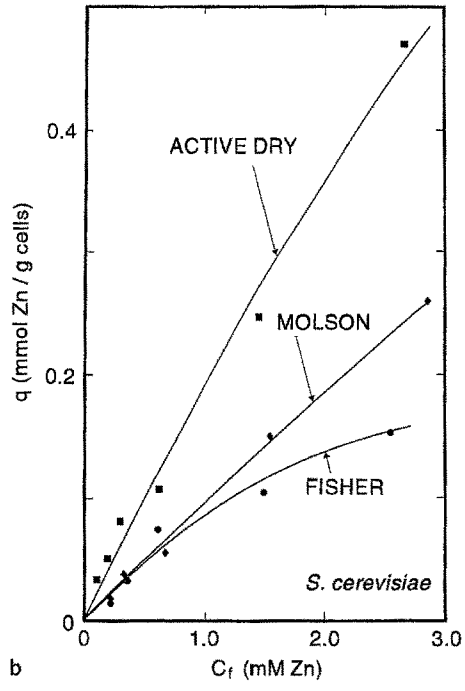



**Fig. 1a–c** Effect of buffering on metal uptake by *Saccharomyces cerevisiae* (McGill) at pH 4.0: ● Buffer present, ◆ buffer absent. **a** Uranium, **b** zinc, **c** copper




a

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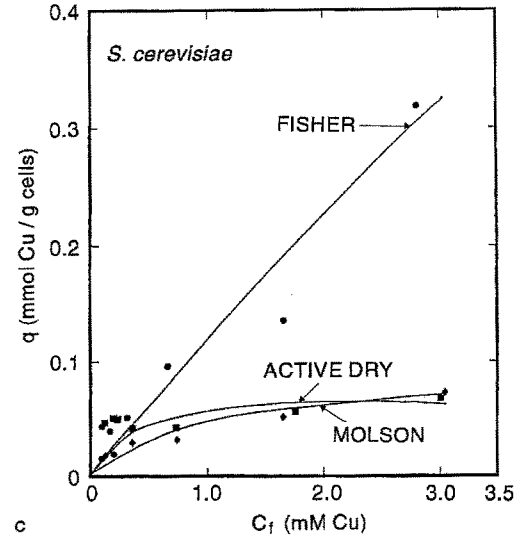



c

capacity for removing copper ions from solution than the other two yeast strains (Fig. 2c).

In summary, dry yeast biomass types examined demonstrated a very high uptake capacity for uranium even at very low concentrations ( $< 0.05$ mM U). The maximum saturation uptake was also reached at concentrations below 0.1 mM U. Dry baker's (Fisher) and brewer's (Molson) yeast biomass types bound larger amounts of uranyl ion when compared to other cations examined. All types of dead yeast biomass also featured steeper slopes of their respective uranium isotherms in the lower concentration regions, indicating higher affinity of these materials for binding uranium rather than zinc or copper.

Dead brewer's yeast biomass (Molson) bound uranium in higher amounts than the other metals. Active dry baker's yeast (Lallemand) exhibited a high uptake of zinc and an extremely low uptake of copper.

## Uptake of metal ions by live strains of brewer's and baker's yeast

For these experiments appropriate wet aliquots of live, washed yeast biomass were used directly it came from the process (Molson Brewery, and baker's yeast producer Lallemand Inc., both in Montreal). The aliquots of wet yeast biomass contained 0.2 g equivalent dry biomass, which was used for calculations of the metal uptake. Sorption isotherms were generated using otherwise the same experimental procedure as for dead dried biomass as a sorbent.

A similar trend was observed for the uptake of uranium by the live yeast biomass as for the dead material. Brewer's yeast (Molson) again sequestered more uranium (60% more as seen in Fig. 3a), also binding it perhaps more strongly, as judged by the sorption isotherm slope, than the baker's yeast strain. However, the live brewer's yeast maximum uranium uptake of 0.234 mmol U/g does not compare favorably to the maximum of 0.592 mmol U/g observed with the dead biomass. Baker's yeasts, on the other hand, showed similar maximum saturation values for both live and dead biomass although the latter had a tendency to bind uranium more strongly according to the isotherm slope.

The sorption isotherms for the uptake of zinc by live *S. cerevisiae* are shown in Fig. 3b. Like dead biomass, baker's yeast took up more zinc (30%) at maximum saturation than brewer's yeast.

Although the maximum saturation was not quite attained, Fig. 3c shows that baker's yeast also removed more copper ions from solution ($q = 0.2$ mmol Cu/g) than did the brewer's yeast strain ($q = 0.16$ mmol Cu/g). This was not observed with the dead biomass where the copper uptake was almost equal and very low for both strains.

**Fig. 2a–c** Biosorption isotherms at pH 4.0 for dry samples of *S. cerevisiae*: ● Fisher dry baker's yeast, ◆ Molsons brewer's yeast, ■ active dry yeast (Lallemand strain). **a** Uranium, **b** zinc, **c** copper

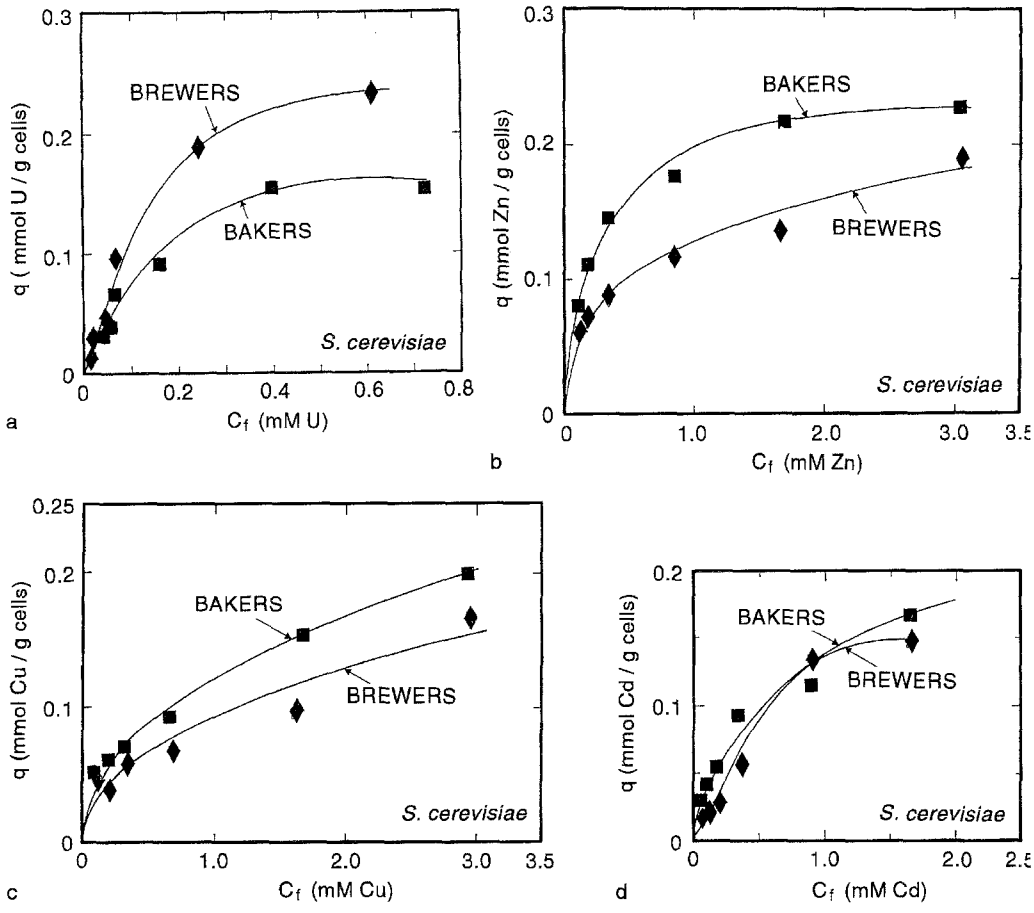



Fig. 3a–**d** Uranium biosorption isotherms at pH 4.0 for live samples of *S. cerevisiae*: ◆ brewer's yeast (subcultured Molson strain) ■ baker's yeast (subcultured Lallemand strain). **a** Uranium, **b** zinc, **c** copper, **d** cadmium

In cadmium uptake (Fig. 3d), the baker's yeast was only very marginally superior, reflecting the same trend as the dead biomass (Volesky et al. 1993).

## Effect of the culture age on uranium uptake

Following a culture period of 12 h and 24 h, a subcultured strain of baker's yeast (Lallemand Inc., Montreal, Canada) was harvested and washed prior to being put in contact with uranyl ions in solution. The younger culture (12 h) in its logarithmic phase sequestered 2.6 times more uranium than the older one. Its biosorption isotherm in Fig. 4a is also steeper, indicating a high affinity of this material for uranyl ions at lower concentrations. Also its biosorptive yield is 52% higher than that of the older culture suggesting that younger yeast cells are more effective in sequestering uranium.

Subcultured brewer's yeast cells harvested at 9 h sequestered three times more uranium than those harvested at 24 h. The isotherms for the younger biomass samples are much steeper than those for the older ones indicating a higher affinity of younger cells for uranium (Fig. 4b).

## *Kinetics of uranium biosorption by live brewer's yeast*

The decrease of uranium concentration in the contact solution with time is shown in Fig. 5 (subcultured brewer's yeasts). The resulting curves share the same common characteristics. Within the first 6–10 min there was a steep decrease in the solution uranium content due to a rapid uptake by the fresh biomass. Within the next 2–4 h the uranium uptake rate gradually decreased. However, small amounts of the metal were being sequestered even during the entire 24-h experimental observation period. It could be said that a true sorption equilibrium was not really established although the longer-term metal uptake rate was almost negligible. Since there was no metabolizable substrate in the solution and the cells were starved, the metal uptake could not have been metabolically mediated.

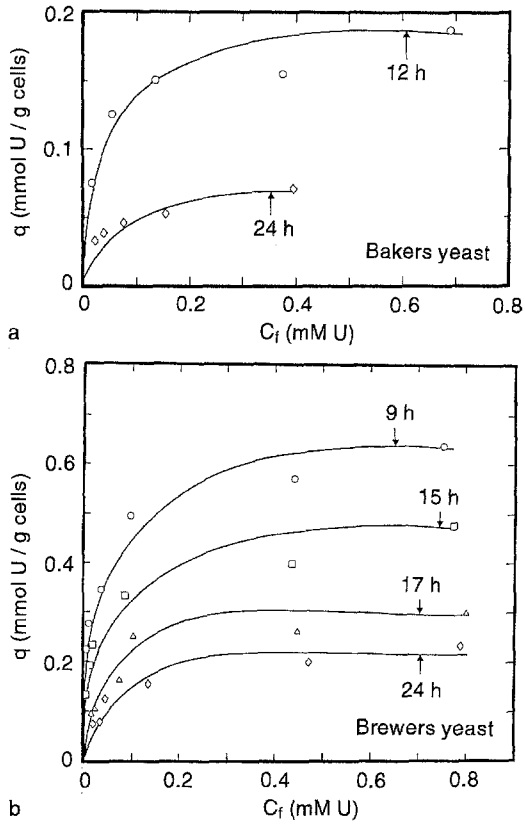



**Fig. 4a, b** Uranium biosorption isotherms at pH 4.0 for live yeast biomass harvested at various times: **a** Baker's yeast (subcultured Lallemand strain): ○ 12 h, ◇ 24 h. **b** Brewer's yeast (subcultured Molson strain): ○ 9 h, □ 15 h, △ 17 h, ◇ 24 h

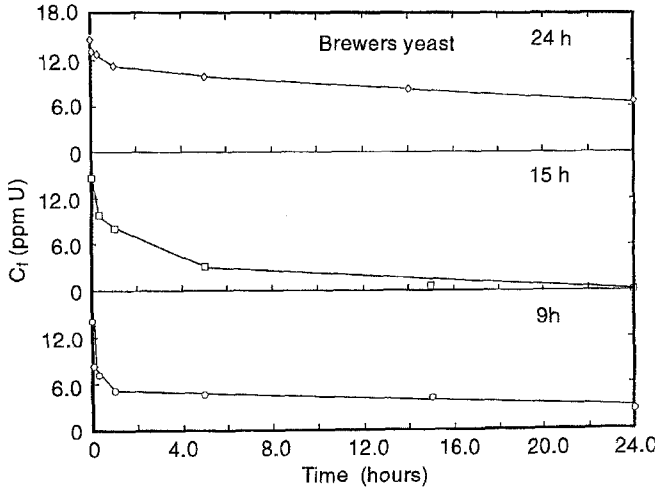



**Fig. 5** Uranium uptake rate curves at pH 4.0 for live brewer's yeast biomass harvested at different times: ○ 9 h, □ 15 h, ◇ 24 h

Residual enzymatic activity, however, could not be entirely excluded.

## Electron microscopy of uranium-laden *S. cerevisiae*

The previous sections have indicated that *S. cerevisiae* takes up uranium to various extents. Brewer's yeast cells demonstrated the highest affinity for uranium and have been examined by transmission electron microscopy to reveal the location of the metal deposits.

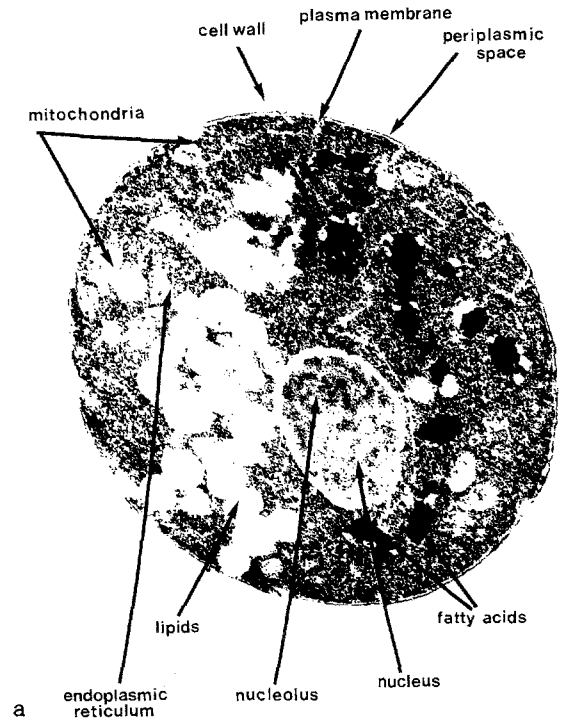
Figure 6a shows a typical yeast cell processed as a control not exposed to uranium. The cell wall appears on the elctron micrograph as a halo surrounding the cell. This is due to the fact that the cells did not contain osmium tetroxide to contrast the cell wall. The native cell contains the regular organelles: the nucleus and nucleolus, endoplasmic recticulum, mitochondria and Golgi apparatus (not seen). Two interesting deposits are seen in the cell, one being electron-transparent lipids and the other, electron-dense fatty acids.

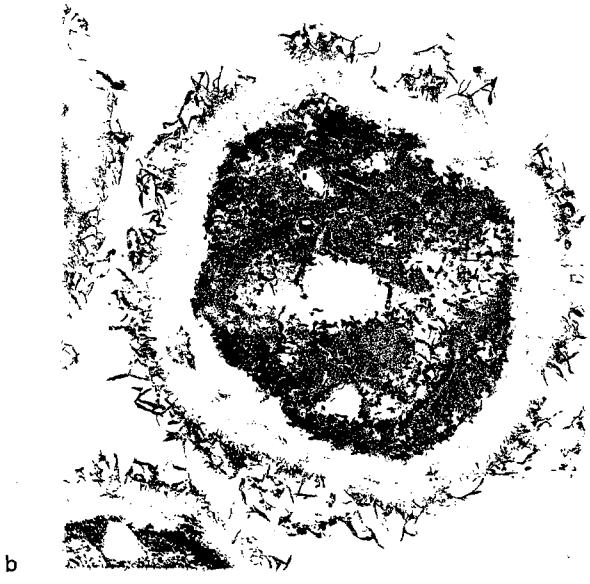
On examination of cells exposed to a uranium solution for 24 h, it is obvious that the uranium deposits demonstrate themselves as crystalline microfibrils about 2 μm long, clearly shown in Fig. 6b. The equilibrium uranium uptake of the biomass sample shown here was 0.088 mmol U/g cells. It is interesting to note that the uranium is deposited on the cell wall surface as well as throughout the cytoplasm of the cell and is not associated with any particular organelle. The structure of the cell is somewhat altered and the components that comprise the cell are not in evidence, the known toxic effects of the uranium exposure perhaps showing. Budding yeast cells exhibit basically the same typical appearance, as demonstrated in Fig. 6c. Again, the uranium is deposited all around the budding cells as well as inside the cell, no organelles are visible, and there are a number of vacuoles throughout the cell. All cells of the exposed sample exhibited the presence of uranium.

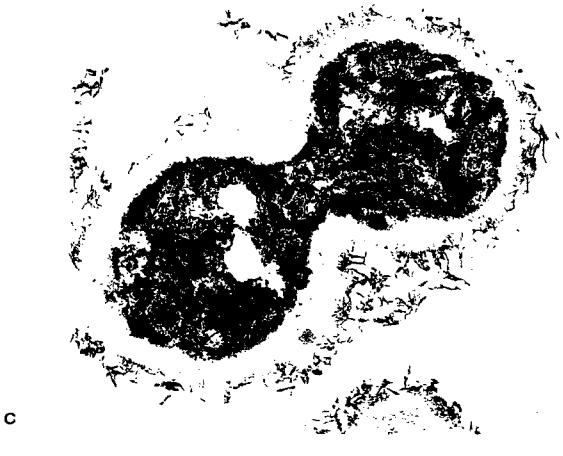
X-ray energy-dispersion analysis confirmed the presence of uranium: M spectral line significantly above background appeared between 3.2 keV and 3.4 keV (not specifically shown). A scan of the spectrum did not indicate the presence of any other metals in the cells at levels detectable by the instrument.

Biomass samples exposed to uranium-bearing solution for different times (15 min, 1 h, 5 h, 15 h and 24 h) were examined, revealing the slow penetration of uranium into the cell interior. Uranium microfibrils appeared on the cell wall and plasma membrane after 1 h and increased after 5 h of contact, as indicated by Fig. 7a, b. The cell began to change appearance with increasing time of contact as more uranium entered. In the last 15–24 h of contact the major cellular organelles could not be detected on the electron micrographs and the presence of vacuoles within the cell increased (Fig. 7c, d). The cytoplasm of the cell started to contract away from the plasma membrane with the cell morphology differing conspicuously from the normal appearance, demonsrating effects of the metal toxicity.

Live and dead cells were examined to determine differences in the cells after contact with uranium.




There was no conspicuous difference between the appearance of uranium-laden live and dead cells. The cell organelles in both cases were not discernible and there were large vacuoles. Uranium was present both on the outside and inside of the cells.

When the cells were washed with acid to determine which portion of the uranium was bound loosely, it was observed that uranium bound to the outer cell wall was washed off whereas the uranium bound to the inside of the cell was not that easily removed.

## Discussion

Biosorption of selected metals was assessed in conjunction with different strains of *S. cerevisiae*, which are particularly widely used in industrial operations. While in-depth study of metal biosorption by the most common yeast would have been beyond the scope of this work, its quantitative estimation has been desirable. The brewer's yeast strain of *S. cerevisiae* possessed the most pronounced ability to remove uranyl ions from solution in both its living and non-living form. The latter performance was comparable to the values obtained for (dead) *R. arrhizus* and other *Rhizopus* species (Beveridge and Koval 1981; Tobin et al. 1984; Treen-Sears 1981). The yeast *S. cerevisiae*, which contains glucan and mannan and traces of chitin in its cell wall, sequestered 0.4 mmol U/g cells at an equilibrium concentration of 0.7 mM. Strandberg et al. (1981) reported uranium uptake of 0.37–0.56 mmol U/g by the bacterium *P. aeruginosa* and the yeast *S. cerevisiae* in 0.37 mM uranyl nitrate solutions, a result very similar to those of the present work. Although dead brewer's yeast biomass bound more uranium than the other metals examined here, the strength of binding of cadmium (Volesky et al. 1993) and zinc was similar, as judged by the initial slopes of the corresponding sorption isotherms, whereas copper was bound less strongly and to a much lesser extent. Baker's active dry yeast (Lallemand) exhibited a high uptake of zinc and an extremely low uptake of copper.

The culturing conditions for the two different strains of *S. cerevisiae* were very different since brewer's yeast (Molson) is normally grown under anaerobic conditions using a malt medium whereas baker's yeast (Lallemand) was grown aerobically on a molasses medium. Significant differences were observed in the uptake of the uranyl ion by the various preparations of the yeast biomass examined. There is evidence that different media as well as growth conditions produce different microbial culture growth characteristics and different

**Fig. 6a–c** Electron micrographs of *S. cerevisiae* cells (Molsons brewer's yeast). **a** A native cell (36 000 ×), **b** a cell exposed to a uranium-bearing solution at pH 4.0 (47 500 ×), **c** a budding cell exposed to a uranium solution at pH 4.0 (28 000 ×)

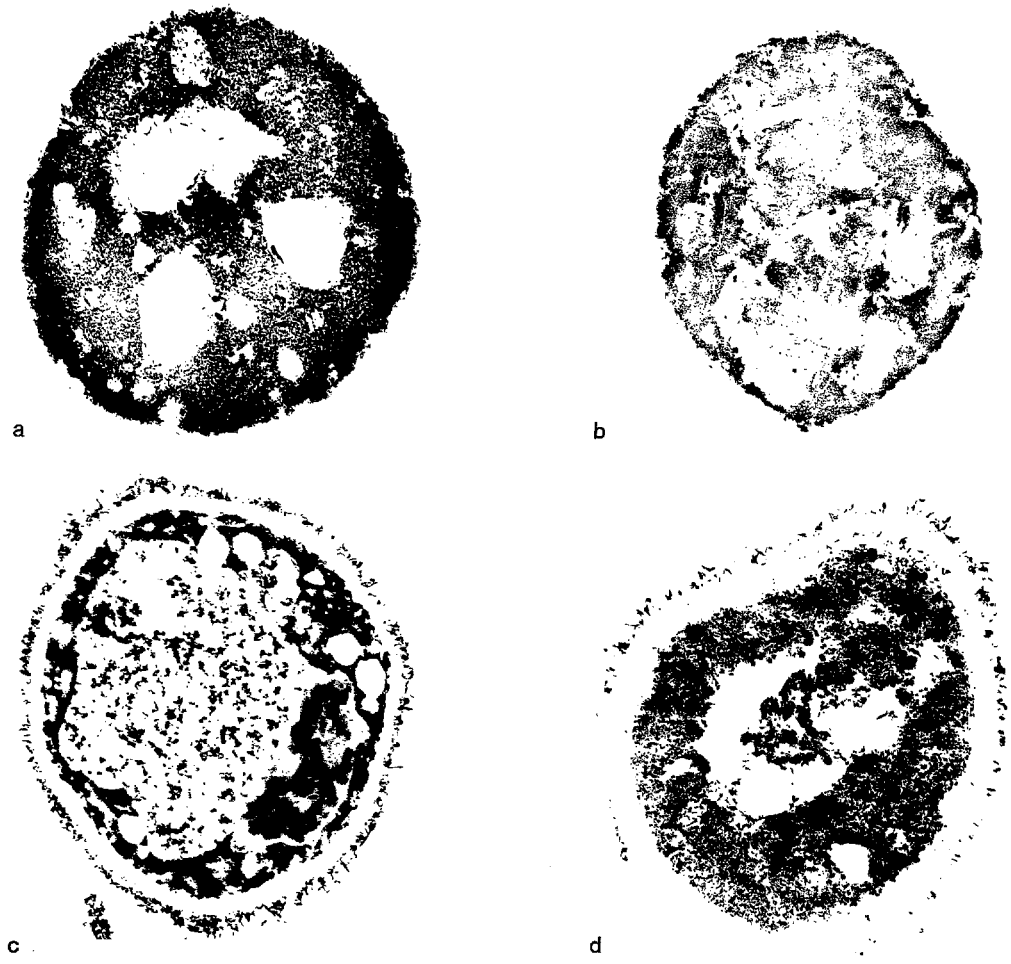



**Fig. 7a–d** Electron micrographs of *S. cerevisiae* (Molsons brewer's yeast) cells exposed to a uranium-bearing solution at pH 4.0. **a** For 1 h; final uptake value of 0.036 mmol U/g cells (46 500 ×). **b** For 5 h; final uptake value of 0.052 mmol U/g cells (27 500 ×). **c** For 15 h; final uptake value of 0.06 mmol U/g cells (25 500 ×). **d** For 24 h; final uptake value of 0.07 mmol U/g cells (26 000 ×)

metal-sequestering abilities (Strandberg et al. 1981; Treen-Sears 1981; Treen-Sears et al. 1984). In this respect it is also important to emphasize that the culture propagation has not been optimized for the purposes of this work and the values of the "biosorbent yeild" are therefore tentative at best. While the culture's "physiological state" may play an important role in the biomass metal-sequestering capabilities, the related data in the literature are either missing or inconsistent, making any comparisons in this regard meaningless. The better sequestering performance of the dried yeast is contrary to the earlier observations comparing uranium uptake by "native" and "dried" *Penicillium chrysogenum* (Jilek et al. 1978).

Baker's "active dry" yeast sequestered twice as much zinc as the non-living brewer's yeast. The binding sites for zinc ions on the yeasts are the phosphoryl and carboxyl groups (Ross 1975). It may be feasible to suggest that the different culturing conditions could affect the formation of these groups and hence affect the metal uptake characteristics. While explanation is difficult at this point, it is important to note that the dead cells of both baker's and brewer's yeast sequestered more zinc than the corresponding live cells.

Copper uptake by the dead biomass of baker's and brewer's yeast was very low, with brewer's yeast sequestering a little more copper than baker's yeast. The live strains, on the other hand, sequestered three times as much copper as the dead species. Norris and Kelly (1979) reported 0.163 mmol Cu/g cells for live yeast *S. cerevisiae*. Significantly higher copper uptake by the live yeast biomass implies a possible active copper-binding mechanism. There is some evidence that *S. cerevisiae* contains a copper-binding protein similar to metallothionine (Rupp et al. 1979). Copper uptake by the live *S. cerevisiae* strains seems to be fairly independent of the strain and culture conditions.

Unfortunately, more specific conclusions concerning the differences between passive metal biosorption and, potentially metabolically mediated, active metal bioaccumulation or even repulsion by living yeast cells

cannot be drawn from this exploratory and limited piece of work.

Only brief electron-microscopic observations were made with brewer's yeast biomass, which exhibited the highest uptake of uranium. Uranium-laden brewer's *S. cerevisiae* cells showed that sequestered uranium was deposited in the form of crystalline microfibrils about 2 μm long on the outside of the cell. While Strandberg et al. (1981) reported the same observation, their biomass was not exposed to uranium solutions for more than 2 h. Rothstein and Hayes (1956) proposed earlier that the uranyl ion forms a relatively stable but reversible complex with phosphate or nucleic acid groups on the surface of the yeast cell, without entering the cell. They showed that acid washing of the cells easily removed the uranium deposits formed on the outside of the cells. In the present study the uranium was observed also deposited within the cell after a prolonged exposure of up to 24 h. All the *S. cerevisiae* cells viewed after the 24-h contact time contained uranium, while Strandberg et al. (1981) reported an unexplained peculiarity that only 32% of the cells did. The time of contact could also account for this difference.

The chemical state of the metal in the sorption system, e.g. cationic or hydrolyzed neutral/anionic species, may obviously play an important role in biosorption (Darimont and Frenay 1990). Uranium, which has been shown to biosorb so well in many investigations, occurs in an ionic state with a relatively large ionic radius, making it more amenable to (bio)sorption (Tobin et al. 1984).

While uranium biosorption kinetics has not been studied in appropriate depth with *S. cerevisiae*, this work confirms recent indications (Gadd et al. 1988; Khalid et al. 1993) that the bulk of it happens very fast, reaching almost 70% of the final uptake value within the first 15 min of contact. Further deposition of the metal occurs slowly over a period of many hours. However, cadmium uptake by the same microorganism was shown to be even faster, attaining 73% of the equilibrium value within the first 3 min of contact (Volesky et al. 1993). There is a direct relationship between the time of contact of the living cells of *S. cerevisiae* with uranium and the morphological changes observed in the cell. High concentrations of uranium are reported to inhibit cell metabolism as well as exert toxic influences on the cells of *S. cerevisiae* (Rothstein and Hayes 1956). This would explain the major changes occurring throughout the cell. Both living and nonliving cells of *S. cerevisiae* exhibited a similar tendency to bind uranyl ions to both the outside and inside of the cell. This indicates that the biosorption of uranium is not associated directly with vital metabolic functions of the cell. Similarly, Macaskie and Dean (Macaskie 1990; Macaskie and Dean 1985, 1988), working with a bacterial system (*Citrobacter* sp.), observed extensive microcrystallization of uranium and lead from the solution around the cell, which was catalyzed by a phosphatase enzyme acive even after the cell active metabolism ceased.

The same behavior as was observed for the uptake of uranium and zinc was demonstrated with cadmium, that is the dead biomass of baker's and brewer's yeast removed more cadmium from solution than was observed for live cultures (Volesky et al. 1993). Cadmium, an ion which is slightly smaller than uranium, shows a completely different pattern of deposition, as shown by the electron microscopy (Volesky et al. 1993). There was no cadmium deposited on the cell wall; rather there were electron-dense granular deposits within the cell cytoplasm of living *S. cerevisiae*. Not all the cells contained cadmium deposits. The cells were normal in appearance; therefore the concentration of cadmium used did not exert such an acute toxic influence.

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