Bioaccumulation of uranium from waste water using different strains of *Saccharomyces cerevisiae*

Richard Tykva,
Jaroslav Novák,
Eva Podracká,
Karin Popa

Abstract. Five different strains of *Saccharomyces cerevisiae* were tested for their abilities to accumulate uranium from waste water containing competitive ions. Samples of water passing out from a previous uranium mill were used. The strains tested possess different abilities to accumulate uranium. The kinetics of bioaccumulation, the leaching degree, the influence of cell density and their origin were investigated. Under the applied experimental conditions, more than a half of the total activity (uranium and the decay products) could be accumulated after 60 min contact time of 1 mL *S. cerevisiae* suspension and 5 mL of water. The other cations present in solution effectively competed for the uranium accumulation. ^{226}Ra and its decay products were completely retained using all tested strains.

Key words: bioaccumulation • uranium • *Saccharomyces cerevisiae* • competitive ions

R. Tykva, J. Novák, E. Podracká
Institute of Organic Chemistry and Biochemistry,
Academy of Sciences of the Czech Republic,
2 Flemingovo Str., 166 10 Prague 6, Czech Republic

K. Popa✉
Department of Chemistry,
"Al. I. Cuza" University,
11, Carol I Blvd., 700506 Iași, Romania,
Tel.: +40 232 201316, Fax: +40 232 201313,
E-mail: kpopa@uaic.ro

Introduction

Environmental pollution resulting from the release of uranium into soils and natural waters is a serious threat to human and ecological health in many parts of the world. Severe water and soil radioactive pollution occurred in the areas of uranium mining, milling and reprocessing. The mobility of uranium in natural waters is controlled by its partitioning between aqueous and particulate phases, which is in turn affected by aqueous speciation [20]. Temperature and pH, redox conditions, colloid formation and availability of complexing ligands play a role in determining the chemical speciation of uranium [27], and, consequently, its concentration in natural waters.

Various types of (bio)sorbents and coprecipitants were studied to remove and recover radioactive species from liquid waste streams [33]. The biosorption represents a method having excellent metal selectivity compared to the chemical treatment methods [3]. Moreover, it does not produce toxic secondary products [8].

The yeast *S. cerevisiae* has been successfully used for uptake of heavy metals [2, 5, 10, 15, 16, 22, 23, 25, 31] and radionuclides [13, 17, 18, 36]. Analyses were carried out using living [32, 35] or non-living [4, 5, 7, 30, 35] *S. cerevisiae*, respectively for bioaccumulation and biosorption of uranium from low radioactive solutions.

In our previous study [26] we determined, in a model arrangement, the optimal experimental conditions of one

yeast strain type for uranium bioaccumulation, without taking in consideration the effect of ionic competition. However, in natural contaminated waters, the uranium series decay products and the non-radioactive cations influence uranium bioaccumulation process [19].

Therefore, the goals of the present study are: (i) to evaluate different strains of *S. cerevisiae* for their ability to bioaccumulate uranium from naturally contaminated water, (ii) to determine the influence of ionic competition using the same strains.

Experimental

Water sample properties and heavy metal analysis

Waste water passing out from the area of a previous uranium mill was used (pH 2.98) having the following concentrations of heavy metals (mg·L^{-1}): Cd 0.0019; Co 0.039; Cu 0.12; Fe 11.0; Hg < 0.0003; Mn 5.3; Ni 0.21; Zn 0.87. Due to the low concentration of uranium in the collected water at the sampling time, uranium in the form of uranyl nitrate hexahydrate (Fluka, p.a., 98.0%) was added to obtain a final concentration of 500 mg U L^{-1}. The activity of the final solution was: 750 Bq U L^{-1} and 250 Bq ^{226}Ra L^{-1}. The ^{226}Ra volume activity was calculated from the activity of the decay products ^{214}Pb and ^{214}Bi in the analyzed sample after equilibrium using a reference source (State Metrological Institute of the Czech Republic, type EB85, 1917.5 Bq).

The heavy metals were detected in solution using a Vista Pro Radial (Varian) atomic emission spectrometer (for Fe, Mn and Zn), an Ultra Mass (Varian) ICP mass spectrometer (for Cd, Co, Cu, and Ni) and an AMA-254 (Altec, Czech Republic) atomic absorption spectrometer (for Hg), respectively. The relative standard deviation in all measurement was less than ± 2.5%.

The uranium activity in water samples was determined by γ-ray spectrometry using an HP germanium detector {[FWHM(1332 keV) = 2.0 keV, relative efficiently (1332 keV) = 25%], amplifier Canberra 2024, ADC Canberra 8077, HVPS Canberra 3106D, MCA Canberra S100, evaluation software Canberra S100}. The relative standard deviation of all measured values was ± 5.0%.

The total volume activity of water was measured using a Beckman LS 6000SE liquid scintillation spectrometer. All spectra were acquired for 600 s, ^{32}P window, using 10 mL of universal scintillator (Rotiszint eco plus, ROTH) and 1 mL of sample solution. The blank sample was prepared by dissolution of 1 mL of distilled water in 10 mL of a scintillator. The relative standard deviation of all measured values was less than ± 5.0%.

Measurement of the ^{226}Ra decay products ^{214}Bi and ^{214}Pb were carried out by γ-ray spectrometry using a high efficiency NaI(Tl) detector connected to a Canberra PCAM-NAI. The ^{226}Ra activity was determined by evaluation software Genie 2000/IPF using the peaks 251.9 keV and 609.3 keV corresponding to ^{214}Pb and ^{214}Bi, respectively [29]. Due to low activity, the measuring time was established for all the samples and standards at 8×10^4 s. The results are the mean from three analyses. The standard deviations (SD) were determined using square root of the sum of squares of the corresponding two ^{214}Bi standard deviations. The relative standard deviation of ^{226}Ra activity was ± 13%.

Origin and cultivation of *S. cerevisiae* strains

Five different strains of *S. cerevisiae* (Sc 21, Sc 79, Sc 87, Sc 89 and Sc 2115) used previously in enantioselective reduction [21] were cultivated under identical conditions. The selected strains obtained from the culture collection of the Institute of Chemical Technology, Prague, had the following origins: Sc 21 is currently produced by a Yeast Company (Kolin, Czech Republic); Sc 79 is used in Pilsner Urquell Brewery (Pilsen, Czech Republic); Sc 87 is used for research purposes; Sc 89 become from the Culture Collection of Yeasts (Bratislava, Slovakia); Sc 2115 was used for different industrial purposes in the Research Institute of Fermentation Industry (Prague, Czech Republic). The yeasts were cultivated in a liquid grow medium [yeast nitrogen base without amino acids 6.7 g·L^{-1} with 1% (w/w) of glucose and 3% (w/w) of Casamino acid] at 28°C for 24 h. The cells were separated by centrifugation (5 min at 10,000 g), twice washed with 10 mL of distilled water and re-suspended in 15 mL of distilled water to analyze accumulation of uranium and other cations. The final suspensions (in stationary phase) contained 1.0 ± 0.05 g of yeast per litter of distilled water.

Bioaccumulation of uranium as well as other radionuclides and heavy metals

The influence of different strains of *S. cerevisiae* was determined by activity measurements. For this purpose, parallel cultures were set up by contacting 1 mL of different *S. cerevisiae* suspension (Sc 21, Sc 79, Sc 87, Sc 89 and Sc 2115) with 5 mL of contaminated water. The experiments were carried out at room temperature (22 ± 1°C), under intermittent shaking. The pH values of solutions after 60 min bioaccumulation did not change too much [2.98 < pH < 3.58]. At a given time intervals (5, 15, 30, 60, 120 and 180 min, respectively), the samples were filtered and the total activity (uranium add its decay products) of 1 mL of the liquid phase was measured in a liquid scintillation spectrometer. Measurements of two different samples were carried out at each time interval and the average value was calculated. Reference sources consisted of different mixtures containing always the same volume of contaminated water as the corresponding samples and distilled water having the same volume as the corresponding cell suspension.

The bioaccumulation capacity was expressed in terms of retention degree (R, %) defined as the activity in yeast at time "t" divided by the activity of the reference sample before bioaccumulation.

At a 60 min contact time, the bioaccumulation degrees of Cd, Co, Cu, Fe, Hg, Mn, Ni, Zn, U, ^{226}Ra, ^{214}Pb and ^{214}Bi were also determined. The applied procedure was identical as that described above. The solutions containing ^{226}Ra and its decay products were closed in glass test tubes for at least 38 days to obtain the decay equilibrium.

The influence of cell density on the uranium accumulation was followed using different suspensions of each tested strain (0.2; 0.4; 0.6; 0.8 and 1.0 g·L^{-1}); 1 mL of each was contacted with 5 mL of contaminated water during 60 min. The experimental conditions were the same as described above.

To determine the amount of radioactive ions retained at the cells' surface, a leaching experiment was performed. After 60 min of accumulation time, the yeast suspension was filtrated and 4 times washed with 2 mL of distilled water. The activity of each washing water was measured.

The bioavailability of each yeast strain was established by the two following experiments:

– Firstly, survival of yeast determination after 60 min bioaccumulation time (for this experiment, the waste water was sterilized; this procedure do not affected the bioaccumulation process). The amount of living cells before and after cultivation was compared as a ratio of the Colony Forming Units (CFU) per mL of experimental suspensions. 50 μL of cells suspension of each strain of *S. cerevisiae* diluted 1:10,000 with distilled water were spread on plates with a minimal medium for yeast (Difco Yeast nitrogen base without amino acids with 1% glucose). Colonies were counted after 2–3 days of cultivation at room temperature (22 ± 1°C). Each value represents the average from two independent measurements. The deviation of this mean value was approximately ± 15%.

– Secondly, measurement of the accumulation equilibrium disturbance after introduction of additional activity into the system. For this purpose, after the bioaccumulation equilibrium was established (60 min after addition), 5 mL of the contaminated water was added to the yeast. The procedure was carried out two times (after 60 and 120 min, respectively). This experiment was performed only for two strains for which we previously observed the best bioaccumulation capacity (Sc 79) and a relatively high capacity of metabolically dependent bioaccumulation (Sc 89).

Results and discussions

The kinetics of uranium bioaccumulation of different strains of *S. cerevisiae* is given in Fig. 1. The obtained results are in agreement with the general mechanism of bioaccumulation of metal ions in *S. cerevisiae* [6, 12] having two steps. The first one is a rapid binding to negatively charged groups on the cell surface and a passive transport of metal ions through the cell wall during a short time (first 5 min). The second step involves penetration through the cell membrane and bioaccumulation of the metal ions onto the cytoplasm.
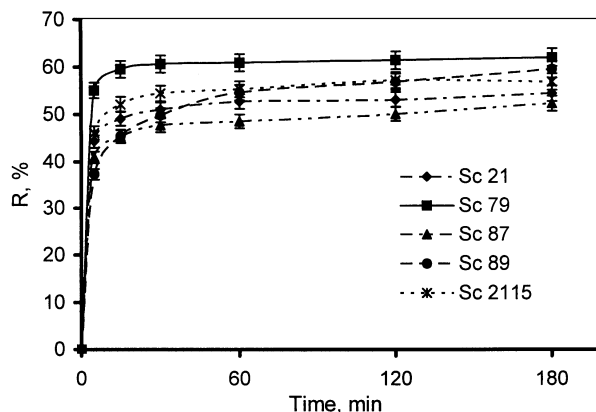

Fig. 1. The dependence of bioaccumulation of uranium and its decay products on the used strains of *S. cerevisiae*.

The equilibrium is rapidly attained for Sc 21, Sc 79, Sc 87 and Sc 2115. Although there are relatively small differences ($\cong 10\%$), the bioaccumulation capacity varies in the two following groups:

Sc 79 > Sc 2115 $\cong$ Sc 89 $\geq$ Sc 21 > Sc 87 after 60 min contact time

Sc 79 > Sc 89 > Sc 2115 > Sc 21 > Sc 87 after 80 min contact time.

The most effective strain was Sc 79, which seems to have the higher capacity of non-metabolically dependent sorption. The involved by-processes could be: precipitation, physical adsorption, ion exchange and complexation [33].

A continuous increase of bioaccumulation capacity of Sc 89 enabled a conclusion that the equilibrium in Sc 89 yeast strain – contaminated water is not established after 3 h and the contribution of cytoplasm bioaccumulation of radioactive ions is higher.

The accumulation capacities of each strain of *S. cerevisiae* toward uranium after 60 min contact time are summarized in Table 1. Although all the strains retained more than a half of the total starting activity (due to the uranium and the decay products), only 30–40% from the soluble uranium is bioaccumulated by the yeast. These differences are due to the ^{226}Ra and its decay products. This study suggests that ^{226}Ra, ^{214}Bi and ^{214}Pb are completely accumulated at lower values of concentration, but this fact could be partly influenced by a very low concentration of ^{226}Ra in the used waste water.

Metal tolerance of fungi has been well documented [11]. The limits of *S. cerevisiae'* tolerance for Cd, Co, Cu, Fe, Hg, U(VI) and Zn was established at 0.4, 8.0, 0.2, 79.0, 0.7, 0.1 and 13.0 mM, respectively [9]. Consequently, the used experimental conditions do not correspond to the toxic domain.

As shown in Table 2, the other cations present in solution interfere in bioaccumulation of *S. cerevisiae*

Table 1. Bioaccumulation of uranium and its decay products of *S. cerevisiae* (after 60 min contact time)

Strain	R (%)				
	Total α + β-emitters	U	^{226}Ra	^{214}Pb	^{214}Bi
Sc 21	53	32	100	100	100
Sc 79	61	39	100	100	100
Sc 87	49	28	100	100	100
Sc 89	55	31	100	100	100
Sc 2115	56	33	100	100	100

Table 2. Bioaccumulation of heavy metals by the used strains of *S. cerevisiae* (after 60 min contact time)
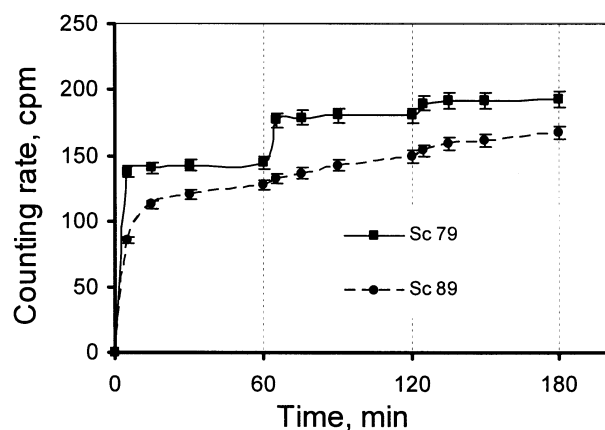
Strain	Final pH	R (%)						
		Cd	Co	Cu	Fe	Mn	Ni	Zn
Sc 21	3.58	21	25	30	43	26	23	42
Sc 79	3.48	10	20	22	44	26	23	40
Sc 87	3.35	21	25	30	26	24	23	20
Sc 89	3.36	26	20	22	40	24	23	48
Sc 2115	3.54	10	25	19	40	24	23	20

strains. Nevertheless, the lowest capacity of uranium bioaccumulation by Sc 87 strain cannot be explained by the preference of yeast for ferric ion at lower pH, as described by Byerley *et al.* [7]. On the contrary, we found that both Fe and Zn ions are less retained by this strain culture. The same phenomenon was observed for Zn bioaccumulation by Sc 2115 strain.

The found differences of bioaccumulation of uranium and/or heavy metals by different strains of *S. cerevisiae* are in agreement with the published results of Park *et al.* [24]. Using two different strains for cadmium bioaccumulation, they found remarkable differences explained by: (i) cell size of the used culture, (ii) specific surface area of the yeast strain and (iii) chemical composition of the outer cell wall (manan layer). Singleton & Simmons [28] described also previously the important key role of cell wall, on which ion-exchange occurs between heavy metal ions and H^+, K^+, Mg^{2+} and Ca^{2+} on the cell wall during the non-metabolically accumulation.

To evaluate the fraction of living cells that survived to the bioaccumulation process, we measured the disturbance of the equilibrium after 60 and 120 min of contact time by adding new amounts of radioactive solution to the yeast. The results (Fig. 2) were expressed in cpm. The biological reply of Sc 79 was found immediately and a new equilibrium was established rapidly. On the contrary, Sc 89 presented a continuous increase of the accumulated activity in time, which was almost independent of the added volume of a radioactive solution. This dependence suggests that the metabolical process is more effective in the case of the latter strain.

In the second step of the bioaccumulation mechanism (metabolically dependent), the radioactive ions penetrate the cell membrane of the living cells going inside the cytoplasm. For example, Volesky *et al.* [34] reported the accumulation of cadmium in vacuoles

Table 3. Amount of living cells (in % of cells at the beginning of experiment)

Strain	Non-contaminated water (%)	Contaminated water (%)
Sc 21	100	100
Sc 79	75	65
Sc 87	100	100
Sc 89	85	70
Sc 2115	100	100

inside the cell as an insoluble phosphate precipitate. However, such a kind of biologically controlled phenomenon is limited only for the living cells. For this purpose, we observed a change of the amount of living cells after 60 min bioaccumulation time and the results were compared with blank samples. The influence of the contaminated and non-contaminated water on tested strains of *S. cerevisiae* proved that two of them (Sc 79 and Sc 89) are sensitive to the used experimental conditions already during 60 min. The decrease of the amount of living cells of these two strains, expressed in percents of living cells at the beginning of experiment was smaller in non-contaminated sterile water than in the contaminated water (Table 3).

The concentration of yeast cells in the accumulation system seems to be an important factor in uranium uptake (Fig. 3). Gadd *et al.* [14] suggested that an increase in biomass concentration leads to the interference between binding sites. For Sc 21 and Sc 87, the increase of the bioaccumulation is a linear function of the cells concentration. The same phenomenon was observed for the three used strain cultures only at small values of the cells density (less than 0.4 g·L^{-1} in the initial suspension). For larger concentrations, an approaching plateau was found. Consequently, it is not necessary to use suspensions with a high cells concentration.

Fig. 2. Influence of the additional activity in the bioaccumulation system.
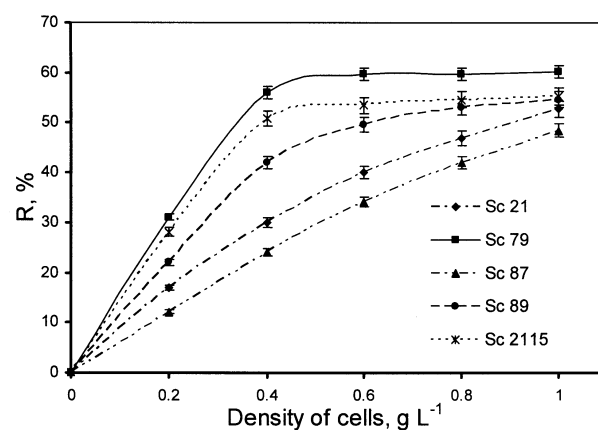
Fig. 3. Influence of the cell concentration on the bioaccumulation degree.

The leaching experiment proved that only a small part (less than a quarter) of the radioactive ions is retained by physical processes. A very low leaching degree of the Sc 89 strain (3.5%) was observed, proving a serious bioaccumulation of the radioactive ions in the cells related to the cell metabolism. This fact is in agreement with the uranium bioaccumulation experiment: it seems that the transport across the cell membrane plays an important role in uranium bioaccumulation. A proposed explanation can be the uranium transport in cytoplasm, mediated by the same mechanism used to convey metabolically essential cations (e.g. K^+, Mg^{2+}, Na^+), as described in [28] for silver uptake.

All investigated five strains were obtained from collections of microorganisms used for industrial or research purposes. Such strains could differ from a natural strain of *S. cerevisiae* not only by their special features desirable for their appropriate application. Their characteristics can be also influenced, e.g., by their survival in non-optimal conditions as water. Water is generally a non-optimal environment for microorganisms. There are a few nutrients whose osmotic pressure of the water is lower than of other liquid environments. In this way, the metabolism and the vitality of microorganisms can be influenced. Similar influences of special features on the survival of microorganisms in particular conditions was observed for recombinant bacteria with new special genes [1] or in the cases of microorganisms with other special features such as resistance to antibiotics or to heavy metals.

Conclusions

The bioaccumulation of uranium, contained in waste water passing out from a previous uranium mill, in five different strains of *S. cerevisiae* was studied. The obtained results proved that the applied yeast strains are differently effective for uranium accumulation.

After 60 min contact time, the accumulation capacity of the tested yeast strains varies in the series: Sc 79 > Sc 2115 $\cong$ Sc 89 $\geq$ Sc 21 > Sc 87. The capacity of uranium accumulation in tested strains of *S. cerevisiae* was affected by the presence of non-radioactive and radioactive competitive cations.

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