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Mechanism of uranium(VI) uptake by *Saccharomyces cerevisiae* under environmentally relevant conditions: Batch, HRTEM, and FTIR studies

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HIGHLIGHTS

- Equilibrium reaches very rapid within 15 min.
- pH shift towards neutral indicates release of hydroxyl ions.
- High ionic strength inhabits biosorption capacity.
- Uptake capacity of heat-killed cells is an order of magnitude higher than live one.
- Electrostatic interaction, precipitation, and complexation are the main mechanisms.

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ABSTRACT

Biosorption is of significance for the safety evaluation of high-level nuclear wastes repositories and remediation of radioactive contamination places. Quantitive study and structural characterization of uranium uptake by both live and heat-killed *Saccharomyces cerevisiae* at environmentally relevant uranium concentration and with different ionic strengths were carried out. Kinetic investigation showed the equilibrium reached within 15 min. In equilibrium studies, pH shift towards neutral indicated release of hydroxyl ions. pH was the most important factor, which partly affected electrostatic interaction between uranyl ions and *S. cerevisiae* surface. The high ionic strength inhibited biosorption capacity, which can be explained by a competitive reaction between sodium ions and uranyl ions. Heat killing process significantly enhanced biosorption capacity, showing an order of magnitude higher than that of live cells. High resolution transmission electron microscopy (HRTEM) coupled with energy dispersive X-ray (EDX) showed needle-like uranium-phosphate precipitation formed on the cell walls for both live and heat-killed cells. Besides, dark-field micrographs displayed considerable similar uranium-phosphate precipitation presented outside the heat-killed cells. The phosphate released during heat-killing process. FTIR illustrated function groups hydroxyl, carboxyl, phosphate, and amino groups played important role in complexation with uranium.

1. Introduction

The activity of microorganisms which are abundant and diverse in the biosphere can alter chemical and physical properties of the environments. Because of high surface-volume ratio, they provide a large contact area with radionuclides. Biosorption and bioaccumulation are main direct interactions between microorganisms and radionuclides, which are of significance for both the safety assessment of high-level nuclear wastes repositories (HLNWR) and remediation of radioactive contamination places [1]. Among the group of actinides uranium is one of the most serious attentions due to its high toxicity and long half-life, hence wide researches have been conducted. *Saccharomyces cerevisiae* is a very ubiquitous biomass type. It can be considered to be a test microorganism because it displays a high metals accumulation capacity of a wide range of heavy metals, and also it is an ideal model microorganism [2] to investigate the radionuclide-microorganism interaction mechanism. In natural environment, both live and dead microorganism present, which are of significance to impact on the migration of radionuclides.

Many investigations aimed at biosorption for removal of heavy metals or radionuclides from waste streams and metals recovery [3–7]. Environmental factors, such as pH, presence of other anions, temperature, and ionic strength can influence metal accumulation by microbes [8–11]. During many environmental factors, pH was the most important one [12,13]. It not only affected protonation or deprotonation of cell wall ligands, but also determined the metal speciation in aqueous solution. Strandberg [14] found

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the biosorption rate and accumulation capacity of uranium by *S. cerevisiae* were subject to environmental factors and the cells could be reused as a biosorbent after chemical removal of uranium. Recently, researches pay more attention to evaluate the important role of microorganisms in metal mobility and to elucidate different mechanisms involved, such as adsorption, precipitation, complexation, reduction, and active transport into the cell [15–20]. Ohnuki [21] declaimed a important uranium(VI) uptake mechanism by *S. cerevisiae*, suggesting that the yeast's cell surfaces offered the specific conditions for U(VI)-phosphate mineralization process. Simonoff [22] reviewed uranyl could be reduced to uraninite by many microbes and hence microorganisms can help to fix or limit the spreading of radionuclides. To explore uranium accumulation by *S. cerevisiae* under culture conditions, Liu [23] showed the *S. cerevisiae* growth curve did not be markedly inhibited by uranium below concentration of 300 mg L^{-1}.

However, few works are available on the quantitive study and structural characterization of uranium uptake by both live and dead *S. cerevisiae* cells especially at environmentally relevant uranium concentration and under different ionic strength. To our knowledge, this is the first work that aims at comparing distinctive accumulation capacity of live and heat-killed cells and exploring possible mechanisms on uranium uptake by *S. cerevisiae* under environmentally relevant conditions. In this work, kinetic and equilibrium studies were carried out. In equilibrium study, dry biomass dose, pH, ionic strength effects as well as the influence of cell activity status were discussed. To characterize bound uranium by *S. cerevisiae* and understand mechanisms involved, high resolution transmission electron microscopy (HRTEM) coupled with energy dispersive X-ray analysis (EDX), Fourier transform infrared spectroscopy (FTIR) were employed.

2. Materials and methods

2.1. Culture and preparation of biomass

S. cerevisiae used in this study was procured from Angel Yeast Co., Ltd., China. The Yeast Extract-Peptone-Glucose (YPD) broth was utilized for cell production. This medium contains yeast extract (1%), peptone (2%), and glucose (2%). The cultures were maintained on YPD agar slant. Firstly, the cultures were incubated in flask at 30 °C for 16 h (at 150 rpm). Then, 5 ml of precultures was inoculated into 100 ml fresh medium and reactivated for 30 h. After that, cells were collected by centrifugation at 1700 × *g* for 5 min and washed three times with sterilized deionized water. The biomass suspension was obtained by resuspending the washed cell paste in sterilized deionized water and kept at 4 °C. The dead cells were prepared by autoclaving (at 121 °C, for 20 min). To determine the concentration of the biomass suspension (for both live and heat-killed biomass), a known volume of the suspension was taken out and air-dried for 24 h at 105 °C, using the method of cell dry weight (CDW). Then the refrigerated biomass suspension with known concentration was graded diluted to yield 0.6 or 0.06 g cell dry weight L^{-1} for live and dead biomass, respectively.

2.2. Chemicals

All chemicals used in the experiments were purchased as analytical purity. Uranyl stock solution was prepared by dissolving uranyl nitrate hexahydrate ($UO_2(NO_3)_2{\cdot}6H_2O$) in 1 N HNO_3. The stock solution was graded diluted to provide desired concentration and was stored at pH 2.0. NaOH or HNO_3 solutions were used to adjust pH.

2.3. Uranium assay

Uranium concentrations were determined using the pulsed-laser induced fluorescence method for its high accuracy, availability in wide pH range, interference-free in trace amounts of uranium in solution [24].

2.4. Batch uranium uptake experiments

2.4.1. Kinetic investigation

The kinetic investigation were conducted over a contact time range of 0–4 h. Firstly, 10 ml polyethylene tubes were filled with 0.6 ml of 0.1 mol L^{-1} $NaNO_3$, and then a given volume of cell suspension was added. The mixed cell suspension was pre-equilibrated by shaking for 1 h at 30 °C (250 rpm). After that, 0.6 ml of 10 mg L^{-1} U(VI) stock solution was added. The volume of the mixed uranium cell suspension was kept at 6 ml by adding deionized water. The pH of the mixed system was adjusted to 4.0 with negligible volumes of 1 or 0.1 mol L^{-1} NaOH. At the desired contact time intervals, the cells were separated by centrifugation at 10,280 × *g* for 5 min, and the supernatant was assayed for residual uranium. Cell-free controls were run concurrently in all experiments to ensure U(VI) sorption on the test tube wall was negligible.

2.4.2. Effect of dry biomass dose, pH, and ionic strength

After the mixed cell suspension was pre-equilibrated by shaking for 1 h, 0.6 ml of 10 mg L^{-1} U(VI) stock solution was added. With negligible volumes of 1 or 0.1 mol L^{-1} NaOH, the mixed uranium cell suspensions were adjusted to a pH range of 3.0–7.0. Then the mixed cell uranium suspension was shaken for 2 h. The following steps were the same as kinetic investigation section. To explore one of the factors containing dry biomass dose, pH, and ionic strength effects, the other two were fixed.

2.5. Characterization studies

2.5.1. HRTEM and EDX

In characterization studies, uranium concentration of 100 mg L^{-1} was selected for the need of the employed electronic microscopic or spectroscopic techniques. After pre-equilibration, live or dead biomass (1.6 mg L^{-1}) was incubated for 2 h at 30 °C with ionic strength of 0.01 mol L^{-1} $NaNO_3$. Then the centrifugal collected cells were washed twice with sterilized deionized water and the fresh wet pastes were fixed with 2.5% solution of glutaraldehyde for two days at 4 °C (in 1.5 ml Eppendorf tubes). After treated with OsO_4, they were dehydrated by 15 min each time in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 100% ethanol). Following they were embedded in Epon812 and air-dried for 24 h each time in ovens (35 °C, 45 °C, 65 °C). Finally, sections were cut into approximate 80 nm thick and mounted on copper-nickel grids. Transmission electron micrographs were obtained on Tecnai G2 F30 S-SWIN (FEI, USA) at an accelerating voltage of 120 kV.

2.5.2. FTIR spectroscopy

After similar process above, the centrifugal collected cells were washed twice with sterilized deionized water and then dried at 30 °C. The spectra were recorded in a range from 4000 cm^{-1} to 400 cm^{-1} using a KBr window and the pure KBr baseline was automatically subtracted [25]. The spectra were taken on IFS 66 v/S Fourier transform infrared spectroscopy (Bruker Optics, Germany).