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Biosorption of U(VI) by the green algae *Chlorella vulgaris* in dependence of pH value and cell activity

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ABSTRACT

Biosorption of uranium(VI) by the green alga *Chlorella vulgaris* was studied at varying uranium concentrations from 5 μM to 1 mM, and in the environmentally relevant pH range of 4.4 to 7.0. Living cells bind in a 0.1 mM uranium solution at pH 4.4 within 5 min 14.3 ± 5.5 mg U/g dry biomass and dead cells 28.3 ± 0.6 mg U/g dry biomass which corresponds to 45% and 90% of total uranium in solution, respectively. During 96 h of incubation with uranium initially living cells died off and with 26.6 ± 2.1 mg U/g dry biomass bound similar amounts of uranium compared to dead cells, binding 27.0 ± 0.7 mg U/g dry biomass. In both cases, these amounts correspond to around 85% of the initially applied uranium. Interestingly, at a lower and more environmentally relevant uranium concentration of 5 μM, living cells firstly bind with 1.3 ± 0.2 mg U/g dry biomass to 1.4 ± 0.1 mg U/g dry biomass almost all uranium within the first 5 min of incubation. But then algal cells again mobilize up to 80% of the bound uranium during ongoing incubation in the time from 48 h to 96 h. The release of metabolism related substances is suggested to cause this mobilization of uranium. As potential leachates for algal-bound uranium oxalate, citrate and ATP were tested and found to be able to mobilize more than 50% of the algal-bound uranium within 24 h. Differences in complexation of uranium by active and inactive algae cells were investigated with a combination of time-resolved laser-induced fluorescence spectroscopy (TRLFS), extended X-ray absorption fine structure (EXAFS) spectroscopy and attenuated total reflection Fourier transform infrared (ATR–FTIR) spectroscopy. Obtained results demonstrated an involvement of carboxylic and organic/inorganic phosphate groups in the uranium complexation with varying contributions dependent on cell status, uranium concentration and pH.

1. Introduction

Uranium is a radioactive and highly toxic heavy metal which is released into the environment from geogenic deposits and from mining and milling areas by weathering, leaching and anthropogenic activities. It could also enter the environment by an accidental release during nuclear energy production or from storage sites of radioactive waste. The consequences resulting from an eventual mobilization of uranium and other actinides in the environment are under detailed investigation. Comprehensive elucidation of uranium behavior in the environment is necessary for risk assessment of radionuclide migration in the environment and determining effective and economical remediation strategies for contaminated soil and water. Additionally, beside the interactions of uranium with inorganic components, such as rocks and minerals, the biosphere plays an important role in the migration behavior of this element. Many studies have shown results and estimations about possible interactions of uranium with microorganisms like algae, bacteria and fungi (Fomina et al., 2007; Fortin et al., 2004, 2007; Günther et al., 2008; Merroun et al., 2003, 2005; Tsuruta, 2002). Algae represent an important group in the aquatic environment given their abundance and broad occurrence in fresh- and saltwater as well as in soils and extreme habitats (Madigan et al., 2003). Because of their ubiquitous occurrence, the influence of algae on the migration process of uranium in the environment is of fundamental interest. Furthermore, algae stand at the beginning of the food chain and play an economically relevant role as food and food additive. Therefore, the trophic transfer of algae-bound uranium through the food web can be an important process in determining the environmental fate of uranium.

Various studies have shown the unicellular green alga *Chlorella* is capable of binding heavy metals like copper, zinc, nickel, cadmium and lead. This sorption depends on biomass concentration, pH value, temperature, time of cultivation and (bio)availability of the heavy metals (Al-Rub et al., 2006; Cho et al., 1994; Fortin et al., 2007; Fraile et al., 2005; Franklin et al., 2000, 2002; Harris and Ramelow, 1990; Mehta et al., 2002; Sandau et al., 1996). It is known that high amounts of uranium(VI) are bound by *Chlorella vulgaris* in the pH range from 3 to 6 (Günther et al., 2008). Horikoshi et al. (1979a) reported that the sorption of uranium by *Chlorella regularis* is very fast and independent

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of temperature, light, or metabolic inhibitors. This suggests that the sorption of uranium is a metabolism independent process based on physicochemical adsorption on the cell surface.

In addition to physical adsorption, complex formation between uranium and functional groups of the cell surface may occur, similar to the described sorption of cadmium and nickel by *C. vulgaris* (Saitoh et al., 2001). Cell walls of algae are mainly composed of heteropolysaccharides and offer metal-binding functional groups such as carboxyl, hydroxyl, sulfhydryl, phosphoryl and amino groups which cause a negatively charged cell surface (Kalin et al., 2005). Therefore, binding of uranium in its cationic form to the cell surface is in favor over its anionic form, as there are fewer positively charged ligands. Nakajima et al. (1979) concluded, the uranium species UO_2^{2+} and UO_2OH^+ are adequate for biosorption by *Chlorella* in exchange for protons of organic ligands. They also showed that uranium availability for *Chlorella* is hindered by dissolved phosphate and carbonate ions, which form precipitates or stable dissolved complexes, preventing binding to the algal cell surface. The study of Nakajima et al. (1979) also demonstrated that the interaction was not affected by cations, nitrates and sulfates.

In natural habitats living, dead, and decomposed cells occur, all of which are relevant for the prediction of uranium migration in the environment. It is known, that dry or chemically treated cells are more efficient at uranium recovery from aqueous systems than living cells (Horikoshi et al., 1979b). Furthermore, Horikoshi et al. (1981) demonstrated *Chlorella* cells, grown under various culture conditions (autotrophic, mixotrophic), have comparable capabilities for uranium sorption.

In contrast to previous investigations, the present study focuses on the quantitative and structural characterization of the biosorption of uranium(VI) by *C. vulgaris*, especially at environmentally relevant uranium concentrations and in relation to their activity. For the characterization of uranyl species formed in solutions and on/in algal biomass, time resolved laser-induced fluorescence spectroscopy (TRLFS), extended X-ray absorption fine structure (EXAFS) spectroscopy and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy were used. The sorption experiments were carried out with varied uranium concentrations, at different pH values and with living or dead biomass to study the influence of the algal cell status on the uranium sorption.

2. Materials and methods

2.1. Test organism and culture conditions

C. vulgaris Beijernick was purchased from the SAG Culture Collection, (Göttingen, Germany). All experiments regarding culturing and incubation of the algae were performed under sterile conditions. Therefore, media and solutions were autoclaved or in case of heat-labile components sterile-filtered. For preparation of media and solutions all chemicals were used in analytical grade.

Algal cells for all experiments were grown in liquid algal full medium adapted according to Esser (2000) by replacing yeast extract and sodium acetate with 5 g/l glucose and glycine to obtain a defined growth medium. The algae were incubated and stirred in bioreactors (1800 ml or 170 ml) under continuous air supply. The air was filtered by a sterile filter and cultivation was performed following a light/dark cycle of 12 h light/12 h dark until the batch culture reached the stationary phase. Growth was followed by measurement of the optical density at 500 nm with a UV/Vis spectrophotometer (Pharmacia Biotech). Cells for the following experiments were harvested by centrifugation (10,000×*g*, 8 °C) and washed with mineral medium (Kuhl and Lorenzen, 1964). Mineral medium was prepared without ethylenediaminetetraacetic acid (EDTA) and with reduced phosphate concentration (Na_2HPO_4 0.5 µM, NaH_2PO_4 4.5 µM) to minimize uranium complexation and precipitation, respectively. The ingredients of this medium are summarized in Table 1 and compared to those of mining-related waters (Bernhard et al., 1998). Mineral medium supplies all nutrients necessary for survival of the algae during the investigated time span of 96 h, demonstrated by cultivation tests prior to the actual experiment.

Table 1
Overview about ingredients and parameters relevant for uranium speciation of the used mineral medium. Comparison of the used mineral medium with different mining-related waters (Bernhard et al., 1998).

Component	Concentration (mmol l^{-1})			
	Mineral medium	Mine water schlema	Mine water Königstein	Tailing water Helmsdorf
Ca	0.01	6.9	5.9	0.3
Mg	1.0	11.6	0.7	0.9
Na	5.5	20.6	6.1	166
K	10.0	1.0	0.04	0.9
Cl	0.2	3.3	3.8	25.8
Fe	0.025	n/a	n/a	n/a
NO_3^-	10–12	n/a	n/a	n/a
CO_3^{2-}/HCO_3^-	<0.01	3.9	<0.02	10.3
SO_4^{2-}	1.0	20.7	23.9	35.6
PO_4^{2-}	0.005	<0.02	<0.02	0.29
Mn	0.001	n/a	n/a	n/a
Zn	0.001	n/a	n/a	n/a
B	0.001	n/a	n/a	n/a
Cu	10^{-5}	n/a	n/a	n/a
Mo	7×10^{-5}	n/a	n/a	n/a
NH_4	6×10^{-5}	n/a	n/a	n/a
TOC(mgl^{-1})	0.97	62.0	3.5	132.0
U	0.005–1.0	0.021	0.073	0.025
pH	4.4–7.0	7.13	2.6	9.76

Culture purity in all experiments was verified by light microscopy. Dilution series of the algal culture were streaked on agar plates with solid algal full medium (Esser, 2000) to determine the number of colony forming units and by this the number of viable cells at the moment of sampling during the experiments.

2.2. Sorption and desorption experiments

The sorption and desorption experiments with living and dead algal cells were carried out in mineral medium with three replicates. In this study, living and active means algal cells were in a stationary growth phase with 50 ± 10% viable and dead cells, respectively. The freshly harvested algal cells grown in liquid algal full medium described earlier, were washed and resuspended in 150 ml mineral medium (7.3×10^7 cells/ml) with the desired pH value (pH 4.4, 5.3, 6.0, 7.0). To keep algal cells alive and active, the sorption experiments were performed in 170 ml bioreactors as described in Section 2.1. As a control, two extra bioreactors were run under the same conditions during all sorption experiments, one with cells but without uranium and one without cells but with uranium. For the sorption experiments with inactive cells, algal biomass was killed by autoclaving (25 min). The lack of viability was confirmed by plating on agar.

The uranyl nitrate stock solution used in this study was prepared by dissolving solid $UO_2(NO_3)_2\times6\ H_2O$, obtained from Chemapol, Praha/Lachema, Brno, Czechoslovakia, in deionized water. The uranyl perchlorate stock solution was prepared from $UO_2(NO_3)_2\times6\ H_2O$ following the procedure described in (Steudtner et al., 2010). An initial uranium concentration of 5 µM was used to perform the experiments in a more environmentally relevant concentration range and at different naturally occurring pH values. For the test of the sorption capacity of algal biomass and to get a better quality of the spectroscopic measurements described in the following paragraphs, higher uranium concentrations between 0.1 mM and 1.0 mM were applied. Experiments at these high uranium concentrations were only carried out at pH 4.4 to avoid uranium precipitation. The uranium