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Stabilization of Uranium(VI) at Low pH by Fungal Metabolites: Applications in Environmental Biotechnology

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Abstract

Uranium contamination of soils and water is a worldwide problem due to geology or anthropogenic release such as mining, or use of inorganic fertilizers. *In situ* remediation of low and moderately contaminated sites is a complicated procedure due to the complex chemistry of uranium. This study demonstrates that at pH 3.5, a fungal strain isolated from unprocessed uranium bearing shale creates hydrochemical conditions that immobilize 97% of a total of 10 mg L^{-1} dissolved uranium in a 0.20 µm pore system. The redistribution occurred within 10 minutes and remained for five weeks and just 12% of the inventory was retrieved in the biomass. Size exclusion chromatography of the dissolved phase identified organic substances in the range of more than 60 kD down to 100 D as a response to time of incubation. Geochemical modeling indicates formation of uranium-organic complexes where ligand size, coordination chemistry and their tendency to agglomerate determine the redistribution.

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1. Introduction

Uranium has a complicated geochemistry and forms a large number of dissolved and solid species. Under oxidizing conditions, uranium typically is stable as the hexavalent form U(VI) but the element is sensitive to

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changes in pH and redox (pe). In aqueous systems three oxidation states, with different chemical properties, dominate as a function of pe: U^{4+}, UO_2^{+} and UO_2^{2+} where the U(V) is stable only in a very narrow pe-pH window. Uranium (IV) tends to precipitate and become immobile [1] why U(VI) is the most mobile form. Hence, the distribution of dissolved uranium species depends on the pe and pH but also on the presence of coordinating ligands [2]. In acidic environments the uranyl ion (UO_2^{2+}) is a dominant species which has a tendency to hydrolyse and to form strong complexes with carbonate, phosphate and carboxylate ligands [3],[4].

Uranium is present in all aqueous environments, but the concentration range is wide and varies as a function of bedrock, in addition to the hydrochemical conditions. Anthropological sources of uranium pollution can roughly be divided into three main groups: i) from mining and milling operations of uranium ore and reprocessing of waste, ii) from nuclear energy in terms of fuel handling and waste, iii) as depleted uranium (DU) from weapons [5].

Uranium extraction from aqueous phases is typically based on ion-exchange and adsorption by a wide range of adsorbents, such as: ion exchange resins [6], activated carbon [7], zeolite minerals [8], alumina/silica [9], clay minerals [10], talc [11], sometimes in combination with ultra-/nanofiltration [12] and reverse osmosis/electrodialysis [13]. The high environmental cost of uranium contaminated soils in terms of potential toxicity has motivated extensive clean up actions. Such actions are expensive because of advanced technologies. Priority is typically given to highly contaminated sites. For low and medium contaminated soils there is a great need to develop tools which will allow for efficient remediation at reduced cost.

Several biological approaches use fungal biomass as an adsorbent for metals in order to extract them or to control the mobility of the elements. Fungi, yeast, bacteria and algae may enrich uranium by several different physico-chemical and biological mechanisms such as bioaccumulation, biosorption, metal micro-precipitation and chemical transformation [14]-[18]. Uranium-microbe interactions are highly intricate, involving changes in pH and pe, and also complexation and chelation, e.g. by excreted metabolites [19], [20]. The microbial communities excrete a variety of extracellular compounds that serve as ligands e.g. biopolymers, polysaccharides, lipopolysaccharides (LPS) and a number of extracellular melanins [15], [21]. In addition, siderophores could also play an important role in the complexation of metals since several are not specific for iron [19], [21]. Moreover, in most environmental aquatic systems, natural high molecular weight organic matter (i.e. humic substances) constitutes an important pool of ligands, although their interactions with most metals are unknown at a mechanistic level.

The potential use of fungal metabolites for controlling metal mobility by formation of coordination complexes opens a novel possibility for the fixation or removal of uranium from contaminated soils and aqueous systems. This study shows preliminary results from a fungal strain isolated from shale with respect to its use for uranium immobilization by interactions with its metabolites and biomass.

2. Materials and methods

Unprocessed weathered alum shale particles from Kvarntorp, Sweden, approx. size 0.5 x 3 mm, were spread on solid malt extract (Sigma, Germany) agar plates with pH 3.5, adjusted by addition of autoclaved lactic acid. Altogether 30 fungal species were isolated where of nine were selected for further testing and one of them is reported here.

As growth medium for the isolates, liquid malt extract medium was used, whose pH was adjusted to 3.5 by addition of 0.1 M HCl. Only sterile polypropylene vessels (Sarstedt, Germany) were used. Fungal cultures were incubated at 22±1 °C, on an orbital shaker at 150 rpm. After one week of incubation uranium was added to a final concentration of 10 mg L^{-1} as autoclaved uranyl nitrate-6-hydrate. A first sampling was performed 10 minutes after the addition by taking out 15 mL from each culture. Sampling was then done weekly for five

weeks. All samples were filtered through 0.20 µm polypropylene syringe filters (VWR International, USA) immediately after sampling and the filtrates were stored at -20 °C until analysis. After five weeks the biomass was recovered by filtration through 35 µm polyester mesh (Sefar LFM, Switzerland), then washed with sterile de-ionized water (18.2 MΩ), air dried and then stored in a desiccator and weighed. The dry biomass was microwave digested in concentrated HNO_3 (L/S 100) at 160 °C for 30 minutes before analysis of uranium. Electrical conductivity and pH were measured with standard electrodes (Radiometer CDC866T and Metrohm 6.0257.000, respectively). The size distribution of dissolved organic compounds was evaluated with size exclusion chromatography using an Agilent Bio SEC-5 column. The mobile phase was a 50 mM phosphate buffer at pH 6.8 and detection was made at 210, 225, 250, 365 and 600 nm. Calibration of the signal was made with polyvinyl sulphonate standards in the range 1.2 to 60 kD. Analysis of uranium was performed with an Agilent 7500cx ICP-MS using the Merck VI multi-element standard solution for calibration.

Table 1. Uranium concentrations in aqueous phase and dry weight biomass after five weeks of incubation

Time of incubation	Concentrations of dissolved U in the aqueous phase (µg L^{-1})	Decrease of dissolved U in the aqueous phase (%)	U adsorbed to biomass (µg g^{-1})	U adsorbed to biomass (%)
10 min	302	97.0		
1 week	437	96.6		
2 weeks	404	96.8		
3 weeks	372	97.1		
4 weeks	290	97.1		
5 weeks	368	97.1	61.5	11.7

3. Results and discussion

3.1. Uranium concentration and dissolved uranium species in aqueous phase and uptake by fungal biomass

In the following sections, the term sorption is used to denote the difference between the total initial concentration of uranium and the concentration in the filtered (0.20 µm) solutions. The latter is referred to as the "soluble" fraction. As shown in Table 1. uranium sorption at pH 3.5 was rapid and within 10 minutes 9.7 mg L^{-1} of the initial 10 mg L^{-1} had been lost from the solution. Thus, a rapid uptake by the fungi, sorption to hyphae or colloidal exudates are possible mechanisms. Considering the velocity of the process, surface processes are most likely. The amount that remained in the dissolved phase corresponds to 3% of the inventory. Of the latter fraction only some 12% was recovered upon digestion of the hyphae, which support that the rapid redistribution is controlled by surface processes (Table 1). After the fifth week of growth the uranium uptake by the biomass reached 61.5 µg g^{-1}. These results indicate that bioaccumulation of U(VI) is not an efficient mechanism at pH 3.5 for the strain under study. Similar results were explained by the high proton gradient across the cell membrane [1], [22] which motivates why uranium sorption to fungal biomass decreases with increasing acidity. In fact, at low pH the uranium surface oriented (bio)sorption decreases because of the competition with H_3O^+ for any titrable binding site [23]. However, this is evidently not the mechanism in operation since uranium can be effectively removed from aqueous solution at pH 2-4.5 by fungal biomass of the genus *Rhizopus arrhizus* [14]. A high uranium biosorption capacity of 600 mg g^{-1} dry weight was observed in *Trichoderma harzianum* for initial uranium concentrations up to 1100 mg L^{-1} [24]. A similar sorption capacity of 650 mg g^{-1} was reported by Kapoor et al. [25] by immobilized *Aspergillus niger*

powder beads. For dead dried biomass maximum capacities of 162 mg g^{-1} for *Acremonium sp*. and *Aphanocladium sp*. when exposed to 300 mg L^{-1} uranium [26], and around 98 mg g^{-1} for *Aspergillus fumigatus* beads with 200 mg L^{-1} in the solution [27] were observed. It must be noted that in these experiments the uranium concentration was 10 mg L^{-1}. For this reason, the uptake to the biomass did not reflect its maximum capacity. It is clear, however, that this strain can act as a sorbent for uranium.

3.2. Fungal metabolites

Size exclusion chromatograms recorded at 250 nm (Fig. 1.) show the molecular weight distributions of organic molecules in the filtered (0.20 μm) aqueous phase after five weeks of fungal growth in media where U(VI) had been added as the nitrate. Under the present analytical conditions the calibration range is 1.2 kD to 60 kD which corresponds to a retention time window of 5.6 to 12.5 minutes. Molecules with sizes outside of the calibration range are denoted by their retention times although this particular column separates reasonably well down to 150 D. The SEC analysis of all three samples (control media, fungi with uranium after 10 minutes and five weeks, respectively) shows the same size distribution of the compounds within the calibrated time window. These peaks are not base line separated why the polydispersity is high for all peaks but not calculated at present. Identification of these compounds is in progress. The chromatograms also contain a series of compounds with retention times below the lower cut off of the column. Concerning the intensities of the signals (Fig. 1.) there are some features of interest. In the growth medium the peak T12.4, T13, T16 and T17.5 minutes increases when the fungi is present, which indicates that these compounds are produced during the time of incubation. Peak T11.5 indicates a production of organic compounds which after five weeks are consumed. At retention time T15 minutes there is a time dependent consumption of compounds present in the medium that might be related to the growth of the fungi. Perhaps the peak at T14.2 minutes is the most interesting one since this compound is not present in the medium but produced during fungal growth.

In addition, the presence of uranium induced a pronounced increase in the signal for this compound. This can only be interpreted as an instantaneous formation of a uranium complex with a compound that was present in the medium but could metabolize in the absence of uranium. A more specific characterization of these organic compounds and their uranium content are in progress.

Uranium occurs in surface waters in a variety of physico-chemical forms, including the free metal ion (U^{4+} or UO_2^{2+}) and complexes with inorganic ligands (e.g., uranyl carbonate or uranyl phosphate), and humic substances (HS) (e.g., uranyl fulvate) in dissolved, colloidal, and/or particulate forms. At low pH, uranium mainly exists in the form of the simple uranyl cation UO_2^{2+} [1]. This ion has a strong affinity for a wide range of inorganic as well as organic ligands [28], [29]. The general tendency for the stability of UO_2^{2+} complexes with inorganic ligands decreases in the order $CO_3^{2-} > PO_4^{3-} > SO_4^{2-} > NO_3^{-} > Cl^{-}$ [29]. Considering the low concentrations of carbonate and sulphate ions as well as the weak complexes with nitrate ($UO_2(NO_3)_2(aq)$ $\log\beta = -1.4$ [30] and chloride ($(UO_2)Cl_i^{2-i}$ i =1,2, $\log\beta_1 = 0.17$, $\log\beta_2 = 1.1$ [31]) formation of organic complexes seems more likely. Uranium forms soluble complexes with different stoichiometries with low molecular weight organic acids, such as citrate and oxalate [18], [31]-[33]. From the SEC analysis it is evident that low molecular weight organic matter is present in the dissolved phase. The presence of citric and oxalic acids has been confirmed in the mg L^{-1} range (not illustrated). Hence it seems reasonable that the speciation of soluble uranium is influenced, or probably dominated, by organic ligands. In a "real" aquatic system, the complexation with humic and fulvic acids would have an additional impact [18], [32].

These properties would also indicate that the roughly 97% of the uranium inventory that is retained by the 0.20 μm filter reflects the impact of interactions with similar compounds, although with a size large enough to be retained. Excretion of extracellular metabolites such as high molecular weight organic acids, polysaccharides and a number of proteins as well as polypeptides is taking place in response to the exposure

of toxic metals in their environment. Filamentous fungi secrete considerable quantities of the organic acids such as: oxalic, citric, gluconic, itaconic, and lactic acids [34]-[37]. However, low molecular weight organic acids would not be retained by the filter under ideal conditions but once the pores become clogged smaller particles are retained. Interactions between the matter accumulated on the filter and dissolved species rise as the contact time increases but their quantitative impact is impossible to predict. In this experiment, such interactions could be a part of the explanation behind the high retention of uranium on the filters.

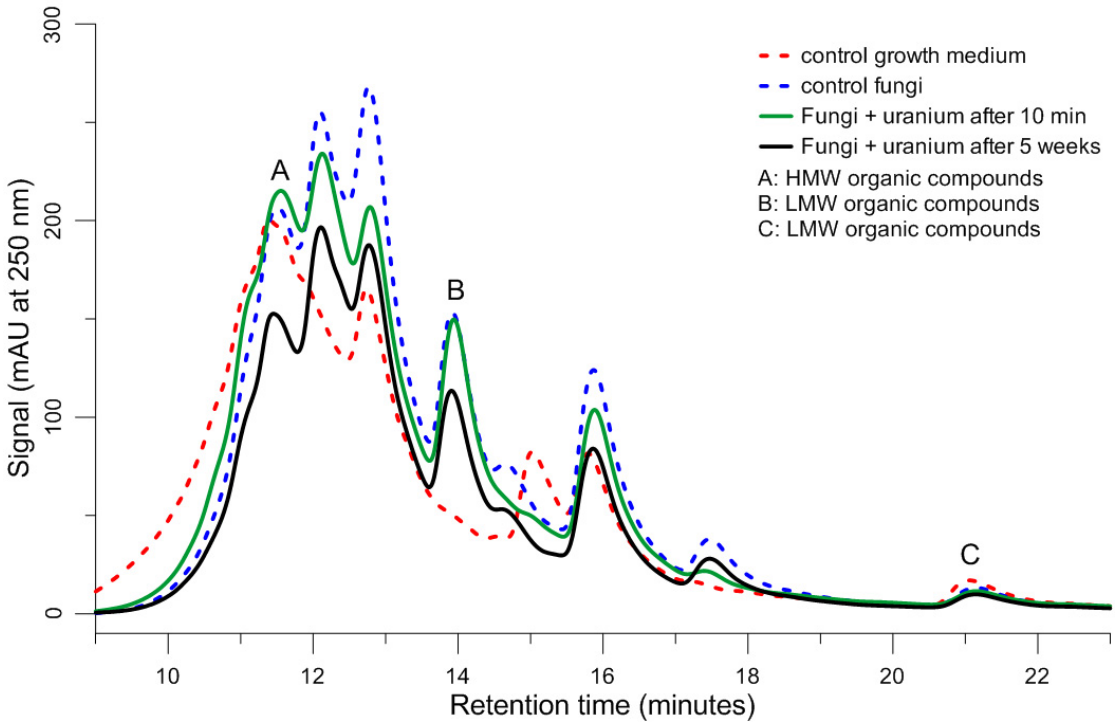

Fig. 1. Analysis of low (LMW) and high (HMW) molecular weight organic compounds with size exclusion chromatography (SEC)

4. Conclusions

This study demonstrates that the activity of a fungal strain isolated from shale prevents some 97%, of a total uranium concentration of 10 mg L^{-1}, at pH 3.5 to pass through pores with a diameter of 0.20 µm. The uranium was added as autoclaved uranyl nitrate. After five weeks of incubation, 12% of the uranium that passed through the pores was retrieved in the biomass. Excretion of hydrophilic organic molecules in the dissolved phase indicates that the redistribution is controlled by these molecules through complexation of the uranyl ions. Additional studies are in progress to identify the nature and properties of the organic matter in the retained phase as well as those that stay dissolved.

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