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Bacteria from uranium mining waste pile: interactions with U(VI)

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Abstract

This study is our first effort to obtain more information on the effects of microbial activities on the mobilization/immobilization of radionuclides in geological environments. We used aerobic and anaerobic strains of bacteria to quantify interactions with U(VI). The quantification of bioaccumulation by two strains of *Thiobacillus ferrooxidans* has shown a slightly higher capability to accumulate U for *T. ferrooxidans* ATCC 33020, isolated from a uranium mine, than for the type strain *T. ferrooxidans* ATCC 23270^T, recovered from a coal mine. The amount of accumulated uranium increased for both strains when the pH was increased from 1.5 to 4.0. Extraction studies with EDTA showed that only a small part of the accumulated uranium is adsorbed on the surface of the cell walls whereas the main part is probably taken up by the cells. We also examined the U(VI) reduction of a sulfate-reducing bacterial strain (*Desulfovibrio desulfuricans* DSM 642^T). In addition, we have studied one sulfate-reducing culture from a uranium mining waste pile (JG 1). Kinetic studies with *D. desulfuricans* have shown that most of U(VI) is reduced during the first 24 h. The yield of this microbial reduction depends strongly on the pH and increases from 10.3 to 99.2% when the pH is increased from 3.1 to 6.2. In nature *D. desulfuricans* strains occur in places where the pH is near neutral.

Keywords: Uranium; Sulfate-reducing bacteria; Iron-oxidizing bacteria; Bioaccumulation

1. Introduction

Bacteria are playing an important role in the transport of radionuclides and other heavy metals in nature [1–6]. Compared to processes involving only inorganic constituents, little is known about bacterial-radionuclide interactions. Microbial activities can cause either dissolution and mobilization or immobilization of uranium in uranium mining waste piles. These interactions can be divided into processes where the bacteria are involved either directly or indirectly. Direct interaction causes oxidation, reduction, accumulation or biosorption of uranium by cells and biopolymers. Indirect interaction can cause a change in pH or E_h of the surrounding chemical environmental system which in turn may result in changes of the uranium system [7,8].

Characterization of bacteria in uranium deposits and mineral heaps have identified acidophilic iron- or sulfur-oxidizing bacteria, in particular *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Thiobacillus acidophilus* and *Leptospirillum ferrooxidans* [9–11]. The maximum concentration and activity of these microorganisms inside the heap was found at 0.25 m below the surface and decreases at points deeper than 1 m. Iron- and sulfur-oxidizing bacteria play an important role in solubilizing uranium from ores. Uranium minerals are often associated with metal sulfides. The oxidation of sulfur and Fe^{2+} results in the production of sulfuric acid and the oxidant Fe^{3+}. The Fe^{3+} in turn oxidizes UO_2 to UO_2^{2+}. The process can be described as follows:

$$4FeS_2 + 15O_2 + 2H_2O \rightleftarrows 2Fe_2(SO_4)_3 + 2H_2SO_4$$

$$UO_2 + 2Fe^{3+} \rightleftarrows UO_2^{2+} + 2Fe^{2+}.$$

This indirect process is predominant in natural uranium ores and it is the basis of bioleaching processes [1–3,9,11–17]. Direct biological oxidation of tetravalent uranium without extraneous Fe^{3+}/Fe^{2+} as electron carriers has also been described in the literature [18,19], but the presence of large quantities of iron sulfides favours the indirect mechanism. Furthermore, *T. ferrooxidans* can accumulate uranium [20] which can be transported and released elsewhere by remineralization. First attempts of an applica-

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tion of *T. ferrooxidans* for bioremediation of uranium-contaminated soils have been presented [21,22].

In addition to autotrophic acidophilic bacteria, in organic matter-rich deeper geological formations heterotrophic anaerobic bacteria, e.g. sulfate-reducing bacteria, may be expected [12]. As known from the literature [23,24], several Fe(III)-reducing bacteria, e.g. *Shewanella alga* [25], *Shewanella putrefaciens* [26] and *Geobacter metallireducens* [24,26], as well as sulfate-reducing bacteria, e.g. *Desulfovibrio desulfuricans* [24,27,28] and *Desulfovibrio vulgaris* [24,29] are able to reduce soluble U(VI) to insoluble U(IV). These strains can use U(VI) as a terminal electron acceptor to obtain energy for growth. Characterization of the precipitate has shown that uraninite (UO_2) is formed during the reduction [27].

The bacterial oxidation and reduction of uranium as well as its accumulation and biosorption play an important role in the geochemical cycle of uranium and must be regarded in the development of remediation strategies. Therefore, we examined the reduction of U(VI) by sulfate-reducing bacteria and the accumulation of U(VI) by acidophilic chemoautotrophic Fe-oxidizing bacteria. For quantification of the accumulation process, we used two strains of *Thiobacillus ferrooxidans* recovered from different environments. While the type strain *T. ferrooxidans* ATCC 23270^T was recovered from a coal mine [30], the other strain (*T. ferrooxidans* ATCC 33020) used in this work was an isolate from a uranium mine [31]. In order to differentiate, whether the main part of the accumulated uranium is weakly adsorbed on the surface of the cell walls or taken up by the cells, we extracted the biomass with EDTA, a strong complex forming agent. To study the uranium reduction, we used a sulfate-reducing strain, *Desulfovibrio desulfuricans* DSM 642^T, recovered from a soil near a gas main, and a culture enriched for sulfate-reducing bacteria (JG 1) recovered from a uranium mining waste pile (Haberlandhalde, Johanngeorgenstadt, Saxony, Germany). These studies included quantitative as well as kinetic investigations under various conditions to yield more information about bacterial reduction which probably has led to the formation of uranium deposits and also could serve as a basis for application in bioremediation processes.

2. Materials and methods

Six hundred ml of the strains *Thiobacillus ferrooxidans* ATCC 23270^T and ATCC 33020 were cultured in medium 2:2 [32] with aeration at room temperature. To dissolve the Fe(III) precipitate which was produced during the growth, we acidified the suspensions with H_2SO_4 to pH 1.3. The bacteria were harvested by centrifugation (12 716$\times g$) and washed three times with 0.1 M H_2SO_4 to remove the phosphate of the growth medium which would form an insoluble precipitate with U(VI). Using two different pH values (1.5 and 4.0), we varied the U(VI) concentration from 1.7 to 26.1 mg/l. The samples contained 220 to 470 mg (dry weight)/ml of biomass. Considering preliminary kinetic studies [20], we incubated them for 2 days on a gyratory shaker. Then the U(VI) concentration in the supernatant was measured by ICP-MS. In the desorption studies, we extracted the biomass with 0.01 M EDTA/0.01 M TRIS solution (pH 7.2).

The sulfate-reducing strain (*Desulfovibrio desulfuricans* Essex6 DSM 642^T) was grown in a bicarbonate-buffered mineral medium with 2×10^{-2} mol/l lactate, 1×10^{-2} mol/l sulfate and resazurin as a redox indicator [33]. JG 1 cultures from the uranium mining waste pile (Haberlandhalde, Johanngeorgenstadt, Saxony, Germany) were isolated and grown in a modification of Postgates medium as described previously [34] with 20 mM lactate as sole source of carbon and energy at the UFZ Leipzig-Halle. The bacterial cells were collected by centrifugation (11 498$\times g$, 10 min), washed three times with NaCl solution (0.9%) and resuspended in 0.9% NaCl solution. For quantification of the bacterial reduction, we adjusted the pH of the samples to 5.0 and incubated them with U(VI) for 3 days on a gyratory shaker. The initial U(VI) concentration was varied from 2.5×10^{-5} to 9.7×10^{-4} mol/l. For kinetic studies with *D. desulfuricans* at pH 3.2, 4.2, 5.0 and 6.1, we used a U(VI) concentration of 1.2×10^{-3} mol/l. The biomass was separated by centrifugation and the concentration of the remaining U(VI) in the supernatant was measured by ICP-MS. In addition to the samples, blank solutions without biomass were prepared and treated in the same way as the samples to quantify the loss of U(VI) by hydrolysis or sorption on the surface of the reaction tubes.

3. Results and discussion

3.1. Accumulation of U(VI) by Thiobacillus ferrooxidans

Fig. 1a shows the uranium concentration accumulated by two different strains of *Thiobacillus ferrooxidans* (ATCC 23270^T and ATCC 33020) at pH 1.5 and 4.0 as a function of initial uranium concentration. The error of the measurements was inside the size of the symbols. For better comparison, the results were normalized to the dry weight of the biomass. The accumulation of uranium is proportional to the external uranium concentration. This is in good agreement with the results observed by DiSpirito et al. [20]. With increasing the pH from 1.5 to 4.0 the quantity of uranium accumulated by the biomass increases slightly for both strains. In comparison to the results of the type strain (*Thiobacillus ferrooxidans* ATCC 23270^T) *Thiobacillus ferrooxidans* ATCC 33020, the strain isolated from a uranium mine, has shown a higher capability of uranium accumulation for both pH-values. In order to clear whether the origin of the strains has an influence on their

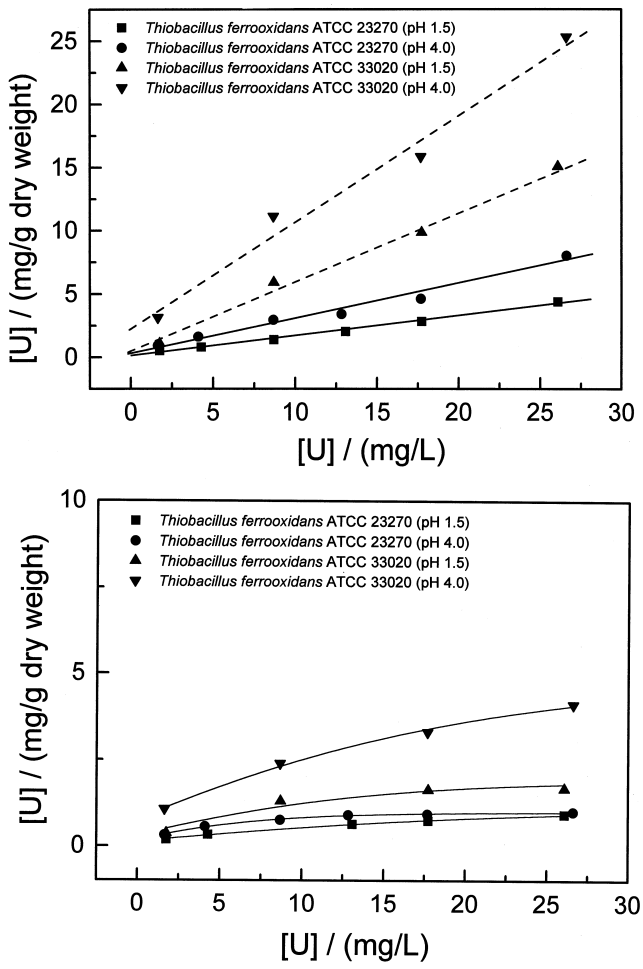

Fig. 1. Total uranium accumulation (a) and uranium concentration adsorbed onto the surface (b) of *Thiobacillus ferrooxidans* ATCC 23270^{T} and *Thiobacillus ferrooxidans* ATCC 33030 at pH 1.5 and pH 4.0 as a function of initial uranium concentration. The results are normalized to the dry weight of the bacteria.

capability to accumulate uranium, further experiments with other strains of *T. ferrooxidans* from different environments as well as with representatives of other *Thiobacillus* species recovered from uranium mines are under preparation in our laboratory.

The desorption studies, using EDTA as a strong complex forming agent to remove the uranium adsorbed on the surface of the cell walls, indicate that the cells take up the main part of the accumulated uranium. Fig. 1b shows the amount of U(VI) that was released from the cells by EDTA treatment. Thereby the term 'uptake' also includes the part of the uranium which is strongly fixed to the cell wall or the cell membrane and cannot be reextracted. As reported in [20], the major cell fractions accumulating uranium in *Thiobacillus ferrooxidans* are the cell wall and the cell membrane, whereas little uranium was detected in the lipopolysaccharide layer, periplasmic material and different cytoplasmic fractions. Our first investigations of U(VI) bound to the biomass with time-resolved laser fluorescence spectroscopy has shown a bathochrome shift of the U(VI) spectrum and a significant increase of the lifetime of fluorescence decay, indicating a possible strong binding of U(VI). The uranium adsorbed on the surface is between 10 and 37% of the total amount of the accumulated uranium while the percentage decreases with increasing initial uranium concentration. For higher uranium concentrations, a limiting value according to maximum saturation on the surface is reached. The graphs shown in Fig. 1b are characteristic of a sorption process.

3.2. Reduction of U(VI) by sulfate-reducing bacteria

Fig. 2 shows the amount of U(VI) removed from the reaction solution by bacterial reduction in dependence of the initial U(VI) concentration for the strain *Desulfovibrio desulfuricans* and the JG 1 culture from the uranium mining waste heap. The samples of the culture JG 1 contained a higher concentration of biomass. Therefore, a quantitative reduction of U(VI) can be observed for initial concentrations up to 220 mg/l. These results have proved that this culture mainly contains bacteria which are able to reduce and precipitate uranium. In contrast to the results of Lovley et al. [27] in our experiments a uranium reduction also occurred without addition of an electron donor. The U(VI) concentrations of the blanks measured by ICP-MS after 3 days did not differ significantly from the initially added concentrations. This indicates that no formation of insoluble hydrolysis species and sorption on the surface of the reaction tubes occurred that may have disturbed the experiments.

The kinetic studies with *D. desulfuricans* were also carried out in NaCl-solution (0.9%) under anaerobic conditions without addition of an electron donor. Fig. 3

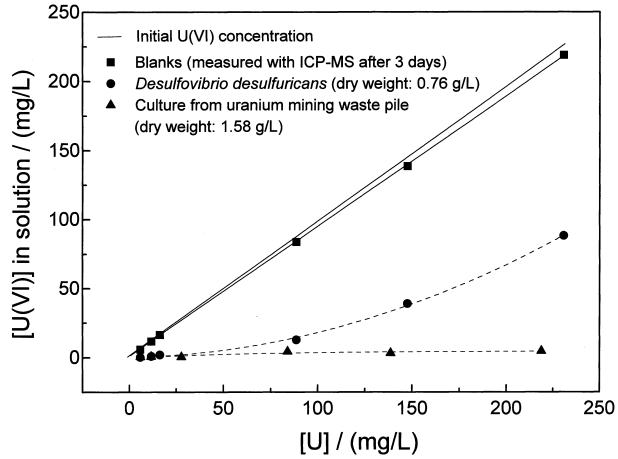

Fig. 2. Decrease of U(VI) concentration in solution after 3 days in the presence of the strain *Desulfovibrio desulfuricans* DSM 642^{T} and the culture (JG 1) from a uranium mining waste pile, and in the absence of biomass (blanks).

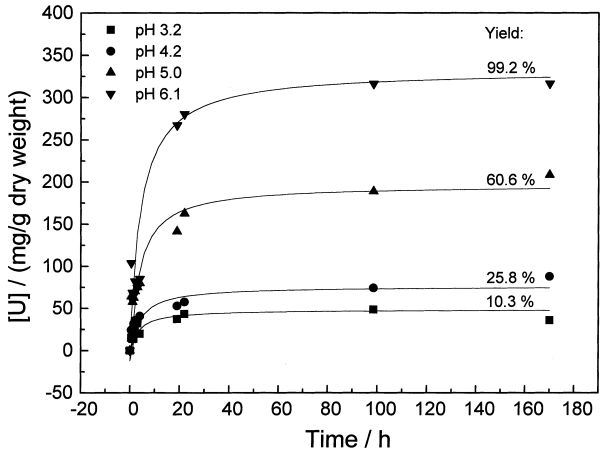

Fig. 3. Amount of U(VI) converted to insoluble U(IV) by *Desulfovibrio desulfuricans* over time at pH 3.2, 4.2, 5.0 and 6.1. The results are normalized to the dry weight of the bacteria.

presents the amount of U(VI) converted to insoluble U(IV) as a function of time for different pH values. The results are normalized to the dry weight of the bacteria. As demonstrated by the time dependencies of the bacterial reduction, the main transformation of U(VI) occurred during the first 24 h in agreement with [35]. From 24 to 100 h only a slight increase of U(IV) was observed. After 100 h, limiting values were reached. An increase from 10.3 to 99.2% was observed when the pH was changed from 3.2 to 6.1, respectively. The increase of the rate and yield with increasing pH corresponds well to the life conditions of this microorganism with a pH-optimum in the neutral pH-range. A first characterization of the precipitate produced by sulfate-reducing bacteria by X-ray absorption near-edge spectroscopy (XANES) has proved the formation of U(IV). Further investigations with *D. desulfuricans* and JG 1 are in preparation.

The information about the interaction of bacteria with U(VI) may help to describe microbiological processes such as bioaccumulation, sorption onto biofilms of minerals or formation of biocolloids as well as bacterial degradation processes (including oxidation and reduction reactions of metals) in natural uranium mining waste piles. The information is also a good base for further investigations with other radionuclides such as Np, Am and Pu that are major environmental contaminants in several countries. A detailed knowledge of the main mechanisms of these interactions will enable a better characterization of migration behaviour of contaminants in natural systems and an extension of the data base for modelling. Furthermore, some of these processes influencing sorption and migration of heavy metals and radionuclides can be used to immobilize the contaminants or to transform them into a less toxic form (e.g. by redox reactions). They can therefore be a basis for developing new remediation strategies using bacteria for transuranium immobilization in waste deposits.

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