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Uranium removal by sulfate reducing biofilms in the presence of carbonates

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Abstract Hexavalent uranium [U(VI)] was immobilized in biofilms composed of the sulfate reducing bacteria (SRB), *Desulfovibrio desulfuricans* G20. The biofilms were grown in two flat-plate, continuous-flow reactors using lactate as the electron donor and sulfate as the electron acceptor. The growth medium contained uranium U(VI) and the pH was maintained constant using bicarbonate buffer. The reactors were operated for 5 months, and during that time biofilm activity and uranium removal were evaluated. The efficiency of uranium removal strongly depended on the concentration of uranium in the influent, and was estimated to be 30.4% in the reactor supplied with 3 mg/L of U(VI) and 73.9% in the reactor supplied with 30 mg/L of U(VI). TEM and SAED analysis showed that uranium in both reactors accumulated mostly on microbial cell membranes and in the periplasmic space. The deposits had amorphous or poor nanocrystalline structures.
Keywords Biofilm; *D. desulfuricans*; uranium immobilization; uranium reduction

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Introduction

In situ microbial reduction and immobilization of hexavalent uranium is considered a possible remediation process for treating uranium contaminated groundwaters. It has been demonstrated that uranium U(VI) can be reduced to U(IV) by planktonic cultures of sulfate reducing bacteria (e.g. Finneran *et al.*, 2002) or biofilms (Roig *et al.*, 1995; Finlay *et al.*, 1999). Recently our research group showed that sulfate reducing biofilms grown on quartz were able to remove almost 90% of uranium, when the concentration of uranium in the influent was 30 mg/L and when the growth medium was buffered with PIPES buffer (Beyenal *et al.*, 2004). However, natural groundwaters are buffered with carbonates, and in such waters uranium removal was considered difficult (Lovley and Phillips, 1992; Fredrickson *et al.*, 2000) because uranium forms complexes with carbonates, thus resisting chemical reduction. The conclusions discouraging use of SRB for uranium removal from groundwaters were reached based on tests run for a short time, and in the presence of high concentration (30 mM) of bicarbonate buffer. However, these conclusions should be verified using a more realistic set of experimental conditions. The goal of our study was to test the ability of sulfate reducing biofilms to remove U(VI) from contaminated groundwaters at concentrations of carbonate that reflected those most commonly encountered in natural waters and for long periods of time, reflecting the conditions relevant to groundwaters.

Sulfate reducing biofilms of *Desulfovibrio desulfuricans* G20 were grown in two flat plate reactors, one supplied with 3 mg/L of U(VI) and the other with 30 mg/L of U(VI) in the growth medium and both buffered with 10 mM bicarbonate buffer. The reactors were operated for 5 months. During that time, we monitored biofilm activity (rates of lactate

and sulfate consumptions) and uranium removal. At the end of the experiment, we determined the chemistry of uranium immobilized in the biofouling deposits using transmission electron microscopy (TEM), energy dispersive spectrometry (EDS), and selected area electron diffraction (SAED).

Material and methods

Two identical polycarbonate flat plate flow reactors (Figure 1) with glass bottoms were used to grow *D. desulfuricans* G20 biofilms. The geometry of these reactors has been described by Beyenal *et al.* (2004). Both reactors were supplied with the growth media of identical chemical composition, except for uranium concentration. The type of growth medium we used was known as metal toxicity medium (MTM) (Sani *et al.*, 2001). It was made of 4.19 g/L of lactate, 2.23 g/L of Na_2O_4S, 0.06 g/L of $CaCl_2{\cdot}2H_2O$, 1 g/L of NH_4Cl, 1 g/L of $MgSO_4$, 0.05 g/L of yeast extract, 0.5 g/L of tryptone, and was buffered with 10 mM of $NaHCO_3$ at pH 7.2. To initiate biofilm growth, the reactors were operated for 3 weeks without uranium in the growth medium. After that time, we added 3 mg/L of uranium to the growth medium of the first reactor and 30 mg/L to the growth medium of the second reactor. Both reactors were operated at the same residence time of 1.2 days.

Uranium concentration in the influent and effluent from the reactors was determined by differential pulse adsorptive stripping voltammetry using cupferron as ligand complexing uranium (Paneli *et al.*, 1995; Wang and Setiadji, 1992). Metabolic activity of the biofilms was computed as the lactate consumption rate, and lactate was analyzed using a lactate concentration measurement kit (Sigma Diagnostic, 735-10). Sulfate concentrations in the influent and effluent were determined by the turbidimetric method (APHA *et al.*, 1975). To determine the chemical nature of the deposits, at the end of the experiment we collected samples of the deposits under anaerobic conditions from each reactor for scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive spectrometry (EDS) and selected area electron diffraction (SAED). The samples were fixed in 2.5% glutaraldehyde then washed with deionized water and dehydrated with ethanol at increased concentration. Finally the samples were embedded using LR white embedding resin and analyzed at Pacific Northwest National Laboratory.

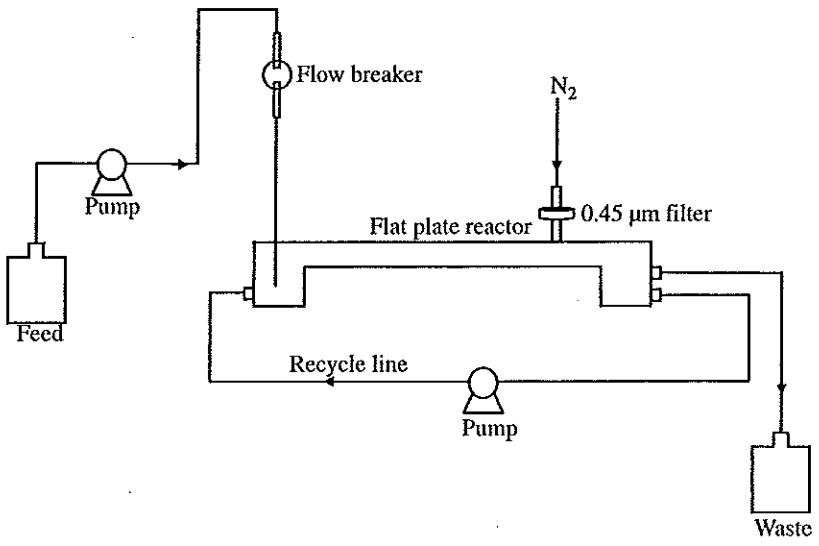

Figure 1 The flat plate reactor

Results and discussion

The amounts of uranium immobilized in both reactors are shown in Figures 2A and 2B. As expected, in the reactor supplied with 30 mg/L uranium, the amount of uranium accumulated in the reactor increased linearly with time. However, the reactor supplied with 3 mg/L uranium did not show the same type of behavior, and demonstrated a sudden decrease in uranium removal after 60 days. This decrease could have been caused either by reoxidation of uranium or, more likely, by a sloughing event in the reactor operated at the lower concentration of uranium. This conclusion remains hypothetical. We did see significant detachment of the biofilm from the reactor supplied with 3 mg/L uranium, but we did not quantify the amount of the detached biomass from the biofilm.

From mass balances in the influent and effluent of each reactor, we determined that the reactor supplied with 3 mg/L of U(VI) removed 30.4% of uranium, while the reactor supplied with 30 mg/L of U(VI) removed 73.9% of total uranium introduced to the reactor. Surprisingly, uranium removal extent and rate were satisfactory in the presence of bicarbonate buffer. However, the residual concentration of uranium in the effluent of each reactor was significant, which could have been caused either by the presence of the bicarbonate buffer, which is a well-known complexing agent of U(VI) (Elias *et al.*, 2003), or by the residence time not being long enough to remove more uranium. Another possibility explaining the high residual concentration of uranium is that the production of hydrogen sulfide was limited by a limiting nutrient concentration. Biofilms in both reactors consumed approximately 95% of lactate and 30% of the sulfate, showing the same

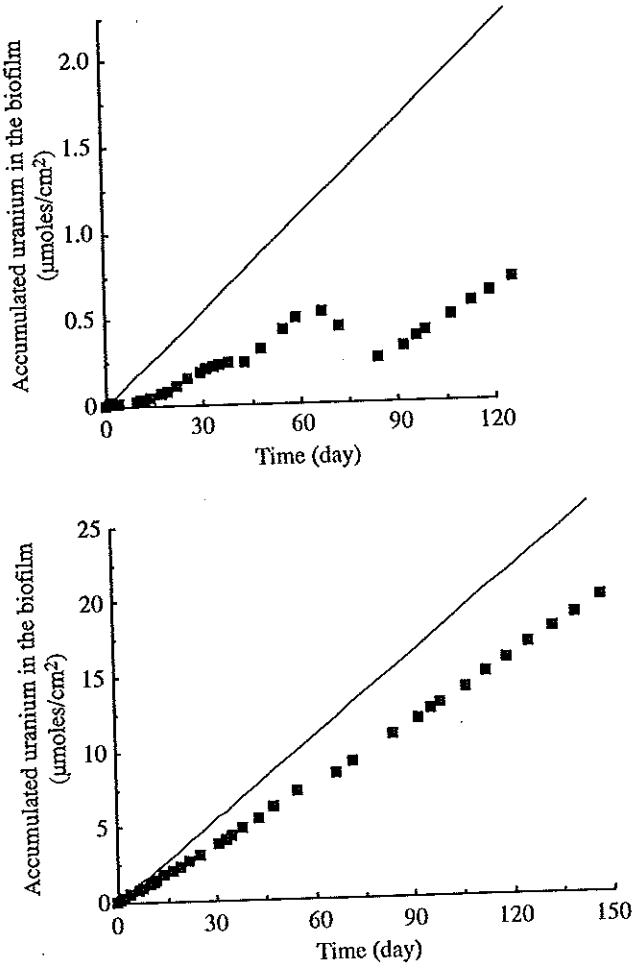

Figure 2 Accumulation of uranium in the biofouling deposits: (A) Reactor supplied with 3 mg/L uranium. (B) Reactor supplied with 30 mg/L uranium

activity in terms of lactate and sulfate consumption rates, and different effectiveness in uranium removal. Even though the reason for the observed differences between the reactors' behavior remains hypothetical, the conclusion that biofilms of sulfate reducing bacteria can immobilize substantial amounts of uranium in the presence of bicarbonate buffer holds.

In the reactor supplied with 30 mg/L uranium, the amount of uranium accumulated in the biofouling deposits was increasing linearly in time. However, the reactor supplied with 3 mg/L uranium did show a more complex behavior, which could have been caused by either releasing of the uranium or by significant sloughing of the biofilm at about 60 days of operation. Sloughing was visually confirmed but could not be quantified experimentally. The continuous line in both figures shows the accumulated amount of uranium introduced to the reactors. The squares indicate the accumulated amounts of uranium removed, computed as the accumulated difference between the amount of uranium in the influent and effluent.

Our conclusion that hexavalent uranium can be effectively removed by immobilizing with microbially generated sulfide hydrogen in the presence of bicarbonate buffer is in disagreement with the results of previously published studies. The differences between our conclusions and the conclusions of the previous studies may be caused by two facts: (1) that we were using lower concentration of bicarbonate buffer, and (2) that we were using longer retention times, and both differences could enhance uranium removal

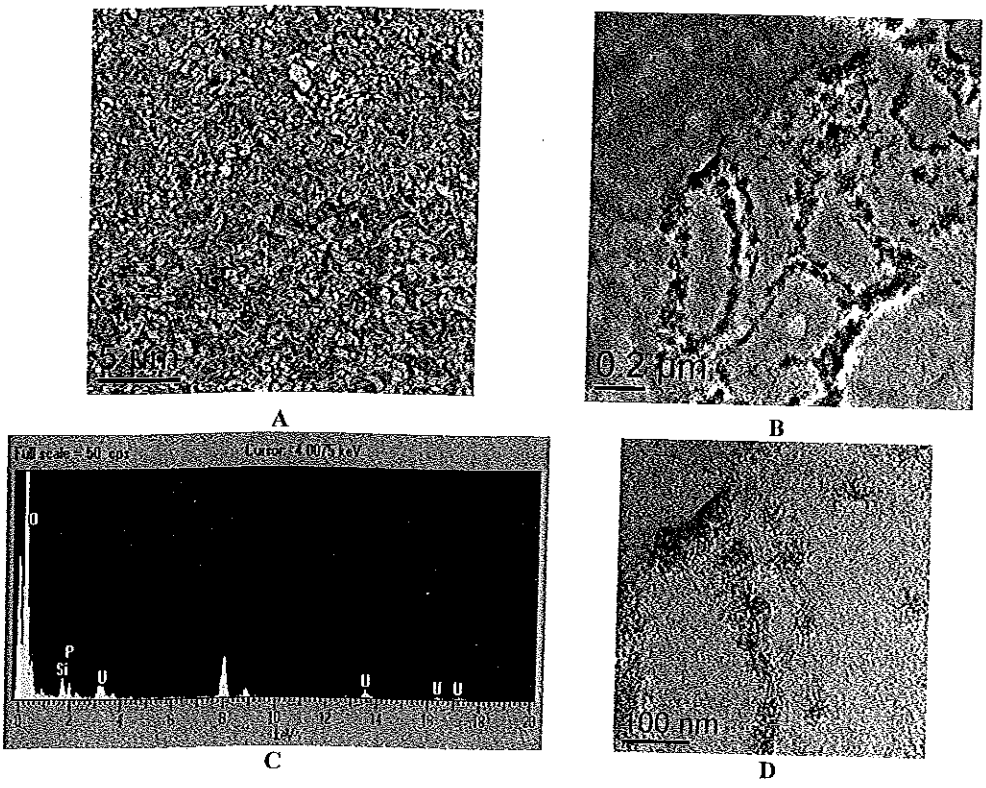

Figure 3 Images of biofouling deposits from the reactor supplied with 3 mg/L uranium. A) Cross-section of biofouling deposits. Low magnification TEM image showing layers of microbial cells that precipitated uranium separated by layers of microbial cells that did not precipitate uranium. B) At a higher magnification the microbial cells show heavy precipitation of uranium on the membranes and in the periplasmic spaces. C) EDS analysis shows that the precipitated deposits contained uranium and phosphates (the peak of Cu is due to the sample preparation procedures). D) HRTEM image of the structure of precipitated deposits containing uranium showing poorly crystalline uranium in needle-like structures

processes. The lower concentration of bicarbonate buffer resulted in increasing the concentration of the non-complexed uranium (remaining in equilibrium with the complexed uranium), which was then effectively immobilized by reacting with the microbially generated hydrogen sulfide, and shifting the equilibrium toward releasing more non-complexed uranium from the uranium-carbonate complexes. This effect could have been amplified by the long retention time in the reactors resulting in substantial amounts of uranium being immobilized.

Figure 3 shows TEM and EDS images of biofouling deposits and of microbial cells in biofilms sampled from the reactor supplied with 3 mg/L uranium. Not all microbial cells in the biofilm immobilized uranium to the same extent. Figure 3A shows a cross-section of the biofouling deposits showing layers of microbial cells which reduced uranium alternated with layers of microbial cells without uranium. The deposits accumulated around the cells (Figure 3B) show that uranium precipitated mainly at the microbial cell membranes and in the periplasmic spaces. EDS analysis (Figure 3C) of the heavy uranium deposit shows that the deposits were rich in uranium, P and Si. SAED analysis indicated that the precipitated uranium was amorphous (results not shown). Figure 3D shows that U(IV) precipitated on microbial cell membranes forming needle-like structures. However, based on crystal structure the uranium precipitate was not uraninite. From our previous experiments these structures showed similar structures to nanocrystalline $U_x(PO_4)_y$.

Figure 4 shows TEM and EDS images of the biofouling deposits/microbial cells from the reactor supplied with 30 mg/L uranium. Not all microbial cells precipitated uranium.

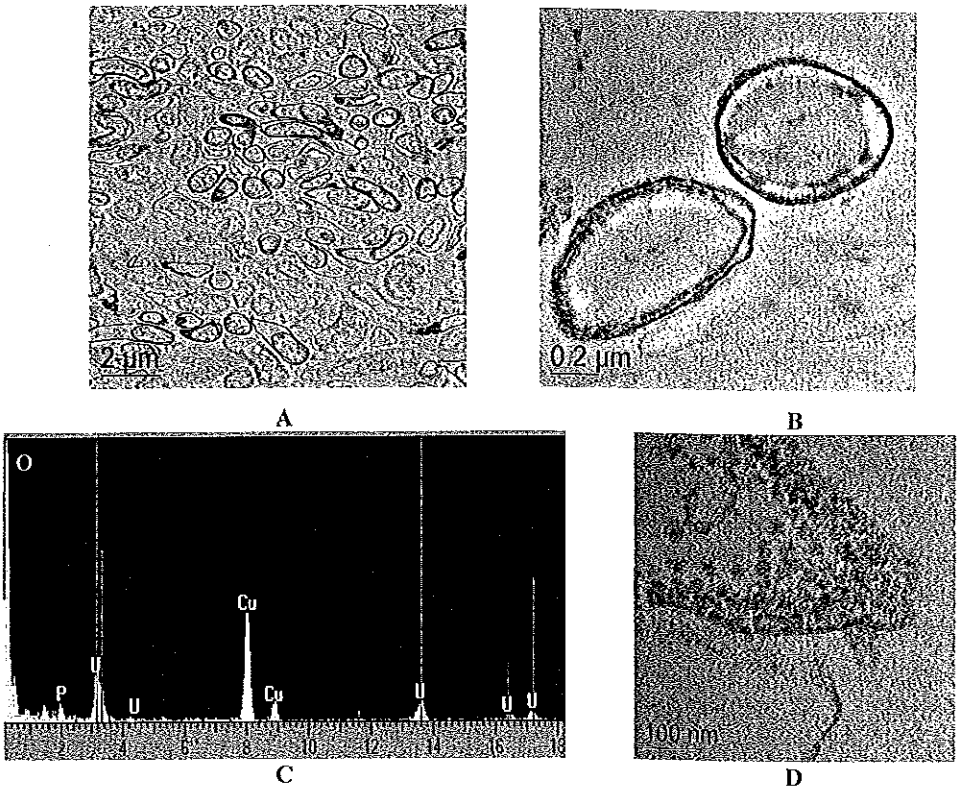

Figure 4 Images of biofouling deposits in the reactor supplied with 30 mg/L uranium. A) Low magnification TEM image shows uranium-precipitating and non-precipitating microbial cells. B) At higher magnification, the microbial cells show heavy precipitation of uranium on the membranes and in the periplasmic spaces. C) EDS analysis shows that the deposits contain uranium and phosphate (the peak of Cu is due to the sample grid). D) HRTEM image of the deposits shows poorly crystalline uranium in nodular agglomerates accumulated on microbial cell surfaces

The TEM image in Figure 4A shows a mixture of uranium precipitating and non-precipitating microbial cells. Figure 4B shows that uranium precipitated on the outer membrane and in the periplasmic space, the same information as in Figure 3B, but slightly more visible. Figure 4C shows an EDS image of an individual microbial cell precipitating uranium and phosphorus. As in Figure 3, the presence of copper is caused by procedures employed in the sample preparation procedure. Figure 4D shows that U(IV) precipitated on the microbial cell membranes as nodular agglomerates with poorly nanocrystalline structure (as determined by SAED, results not shown).

In summary, biofilms of sulfate reducing bacteria reduced and immobilized significant amounts of hexavalent uranium in the presence of bicarbonate buffer. In our flat plate continuous flow biofilm reactors, the efficiency of uranium removal strongly depended on the initial concentration of uranium in the influent, and it was 30.4% in the reactor supplied with 3 mg/L of U(VI) and 73.9% in the reactor supplied with 30 mg/L of U(VI). Despite the differences in removal efficiency between the reactors, the nature of biofouling deposits in both reactors appeared to be the same, as evidenced by EDS, TEM and SAED analyses. Therefore, we hypothetically ascribe the differences in reactor performance to biofilm sloughing events in the reactor operated at a lower concentration of uranium.

Conclusions

- Sulfate reducing biofilms in both reactors immobilized significant amounts of uranium in the presence of bicarbonate buffer.
- TEM images of microbial cells from the biofilm reactors showed a mixture of microbial cells with various uranium depositing capacities, some cells were reducing uranium and some not.
- TEM and SAED analyses showed that uranium accumulated on the microbial cell membranes and in the periplasmic space, and that the uranium deposits had amorphous or poor nanocrystalline structure.

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