An Effective Removal of U(VI) as Bio-Nanoparticles by CN32 Bacterium

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1. Introduction

A variety of anaerobic bacteria can catalyze the reduction of soluble species of U(VI) to insoluble U(IV) forms. Some of these microorganisms, including those capable of S [1] and Fe [2] respiration, do so by a direct enzymatic process coupled to the oxidation of organic compounds or H_2 [3]. The product of this microbial reduction reaction is typically fine-grained uraninite [$UO_{2(S)}$]. U(VI) is readily reduced by Dissimilatory metal-reducing bacteria (DMRB) under anoxic conditions, resulting in the precipitation of uraninite. The rapid rate of U(VI) reduction by DMRB [4] and the relatively low solubility of U(IV) makes biomineralization an attractive option for removing soluble U from groundwaters. The purpose of this research was to investigate the reduction of U(VI) by the DMRB *S. putrefaciens* in the absence and presence of Fe and Mn as alternative electron acceptors for the understanding of the radioactive waste disposal site.

2. Methods

2.1 Bacterium and Medium

S. putrefaciens strain CN32 was obtained from ATCC, USA. CN32 was routinely cultured aerobically in tryptic soy broth (TSB), 30 g/L (Difco Laboratories, USA), and stock cultures were maintained by freezing in 40% glycerol at -80ºC.

Solutions were buffered (Ph ~7) with $NaHCO_3$ at 30 mM. Sodium lactate was added as the electron donor in 10 mM. Medium was dispensed into tubes, purged with N_2 gas for $NaHCO_3$-buffered medium and sealed with thick butyl rubber stoppers.

CN32 cells were harvested at mid to late log phase by centrifugation from TSB cultures, washed with buffer to remove residual medium, suspended in $NaHCO_3$, and purged. Cells were added to buffers to obtain a final concentration of 8 mg/L protein.

2.2 Bacterial Reduction Experiments

The ability of *S. putrefaciens* strain CN32 to reduce U(VI) was evaluated in the presence or absence of other inorganic elements such as P, Fe, and Mn. In a typical experiment with 100 mL final volume, 1 mL of CN32 was added as 8 mg/L protein, followed by $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), KCl (1mM), Na_2HPO_4 (0.3 mM), $FeCl_2$ (0.2 mM), and $MnCl_2$ (0.2 mM). The concentration of injected U was 5×10^{-5} M. All bacterial experiments were incubated at 30ºC with shaking at 120 rpm for 2 months, while cells were metabolically active.

3. Results

3.1 U(VI) Reduction and Removal

U(VI) reduction and its decrease in solution were observed in the experiment (Fig. 1). U(VI) reduction was promoted by the CN32 microbe. An influence of other dissolved inorganic cations for the U(VI) reduction and removal was observed by checking solution concentration through an ICP-MS analysis. As the interaction between the bacterium and U proceeds, there was a rapid U decrease. The degree of U removal was much higher in P component in the solution. In contrast, Fe and Mn addition into the reactor decreased the U removal rate.

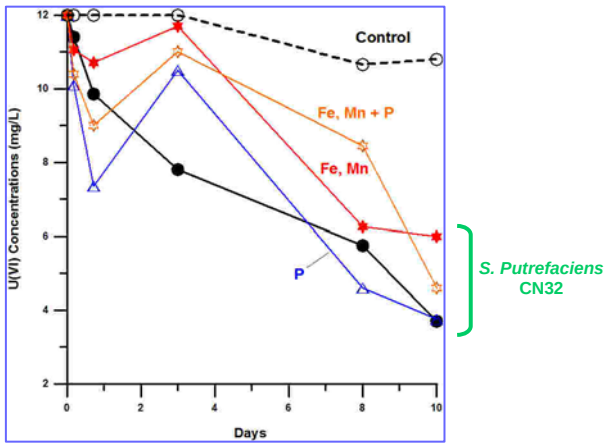

Fig. 1. Changes of U(VI) concentrations by the influence of *S. putrefaciens* CN32 bacterium. There was a large difference for the samples with bacterium and without bacterium (control) to remove U from the solution.

3.2 Influence of Dissolved Fe

Among the added inorganic cations into the reaction bottle, Fe acted as an important factor to affect the U concentration. As the iron exists as Fe(II) in the solution, there was a continuous decrease of U (Fig. 2). However, the decrease of U was not detected in the case of Fe(III).

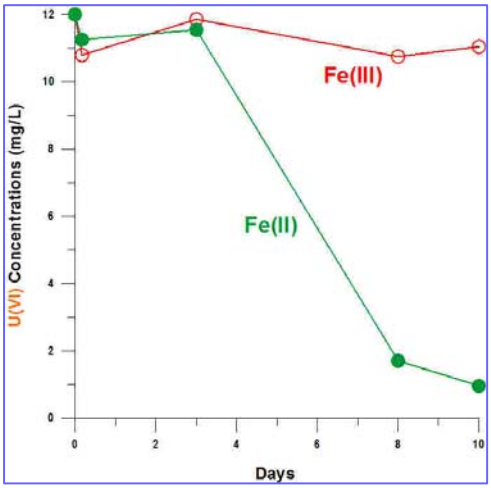

Fig. 2. The removal of U(VI) by DMRB was affected by the oxidation state of iron in the solution.

This means that the oxidation state of iron can give a great influence on the U removal. It can be explained by the transfer process of electrons among bacterium, U, and Fe (Fig. 3). The bacterium usually transfer electrons from organic substance (lactate) to U to reduce U(VI) to U(IV). However, if Fe(III) coexist with U(VI) in the solution, most electrons transfer to iron, instead of U. This indicates that the attraction for electrons by Fe(III) from the bacterium is more favorable than that by U(VI).

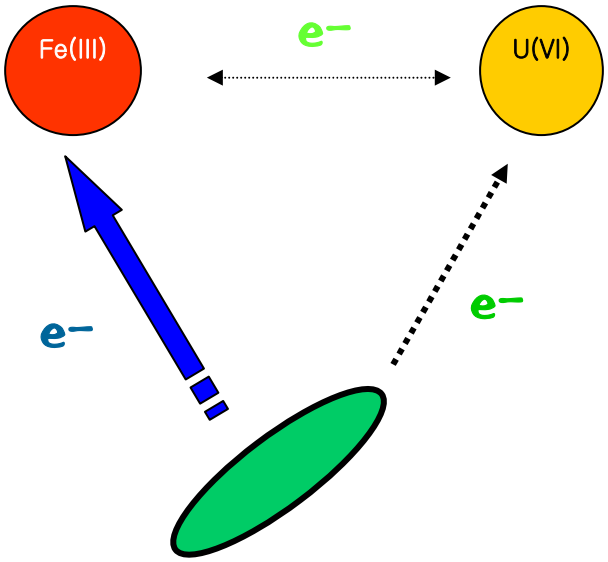

Fig. 3. The preferential reduction of Fe(III) rather than that of U(VI) by DMRB.

4. Conclusions

The *S. putrefaciens* CN32, DMRB, can not only reduce U(VI) to U(IV), but also remove as a form of nano-particles from U(VI)-containing solution. This phenomenon suggests that the anaerobic metal-reducing bacteria living in a deep groundwater can sufficiently reduce oxidized radionuclides forming nano-minerals to retard their movement along the fractures.

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