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Microbially Mediated Removal of Np(V) by *Desulfovibrio desulfuricans* – Implication of Microbial Immobilization at the Radioactive Waste Repository –

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We investigated the capacity of the sulfate-reducing bacterium *Desulfovibrio desulfuricans* to facilitate the removal of Np(V) from aqueous solution. For comparison, we also examined the removal of Np(V) by bentonite under aerobic and anaerobic conditions with sodium sulfide. Np(V) removal (probably after reduction to Np(IV)) was most rapid in the presence of cells, whereas there was little or no removal by bentonite under aerobic conditions. In the presence of cells, Np in suspension declined by 95% or more within 10 h, suggesting that *D. desulfuricans* enzymatically reduces mobile Np(V) to immobile Np(IV) and eventually to stable Np, though chemical reduction by cell metabolite H_2S may contribute. This finding highlights the importance of understanding microbial effects when considering the migration of radioactive elements from waste repositories.

1. Introduction

When considering the safe disposal of radioactive waste containing various transuranic nuclear fuel cycle elements, neptunium (Np) is particularly problematic because its long-lived isotope, Np-237 (half-life, 2.1×10^6 years), is predominant at historical sites.[1] Moreover, in its most stable form, NpO_2^+ is soluble and highly mobile in the environment.

Generally, the reducing form of transuranic elements is immobile in the natural environment, whereas the oxidizing form is more mobile. Therefore, one approach to achieving the removal of transuranic elements from aqueous solution may be to utilize metal-reducing microorganisms to reduce the elements to insoluble and immobile forms. For instance, Lovley et al. demonstrated that uranium (U) can be enzymatically reduced by a dissimilatory Fe(III)-reducing microorganism to an insoluble form, and that this can serve as a mechanism for immobilizing U in sediment and forming U deposits.[2] In that regard, it was recently reported that technetium(VII), a fission product of U-235, is enzymatically reduced to a lower valence by the microorganisms *Shewanella putrefaciens*,[3] *Geobacter mitallireducens*[4] and *Clostridium* sp.,[5] and that Np(V) is reduced cooperatively by the microorganisms *Citrobacter sp*. and *S. putrefaciens*.[6] Thus, certain microorganisms are able to reduce radionuclides enzymatically (direct microbial reaction) and/or chemically by their metabolites (indirect microbial reaction) into insoluble, immobile forms in the environment. Our research has focused on the stabilization and immobilization of Np(V) via microbial removal for safe disposal in subsurface repositories.

2. Materials and Methods

Microorganism. We used the sulfate-reducing bacterium *Desulfovibrio desulfuricans* in this experiment. *D. desulfuricans* (ATCC 7747) was cultured on modified ATCC 1249 medium containing (g/L in distilled deionized water) $CaCl_2$, 0.1; Na_2SO_4, 0.8; NH_4Cl, 1.0; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 2.0; Yeast extract, 1.0; and sodium lactate, added to a final concentration of 60 mM from 60 wt% syrup solution. The pH was adjusted to 7.2. To avoid precipitating iron sulfide during reactions, no ferrous sulfate was added to the medium. After boiling the medium to remove O_2 and then cooling it under O_2-free N_2 gas through a heated copper column, it was pipetted into 10-mL serum bottles that were then aluminum-capped using butyl rubber stoppers prior to sterilization. After autoclaving, filter-sterilized sodium ascorbate and sodium thioglycolate were added to the vials to obtain the reducing conditions necessary for cell growth. The cells were inoculated into each vial under an N_2 atmosphere and cultured at 35 ˚C in an incubator.

Np solution. Np-237 solution in HNO_3 (2 mol/L) was obtained from AEA Tech. PLC, Harwell, UK. For use in the experiments, the original Np stock solution was diluted to a concentration of 5.5×10^{-4} mol/L with HNO_3 (2 mol/L). The oxidation states of Np(V) were confirmed by solvent extraction using PMBA (1-phenyl-3-methyl-4-benzoyl-pyrazol-5-one) as the extractant.[7]

Bentonite. Bentonite mined in Japan was used in the experiment. X-ray diffraction analysis and X-ray photospectroscopy showed that the bentonite used was comprised mainly of montmorillonite and quartz, with various minor components, including pyrite, calcite and beidellite.[8]

Np removal by bentonite under aerobic conditions over a wide range of pHs. To assess the effect of pH on Np removal by bentonite under aerobic conditions, 5 mL of 0.5 g/L bentonite suspension, with 0.01 M $NaNO_3$ as a background electrolyte, were added to 10-mL glass serum vials, after which Np solution was added to a final 1.0×10^{-6} mol/L, and the pH was adjusted with HNO_3 and NaOH. The vials were then stoppered with cotton to allow equilibration with atmospheric CO_2 and shaken at 35 ˚C. After 24 h, pH and Eh (vs. NHE) were then measured using a pH-ORP meter (TRX-90Si, Toko Co., Japan). The suspensions were then filtered through a 0.22 μm filter (Millipore), and the filtrate was subjected to Gamma-spectrometry (GL2020S, Canberra Inc., USA) to determine the Np concentration, which was measured as the spectrum peak area counted at 92.29 keV.[9] The amount of Np adsorbed (i.e., solid phase) was calculated as the differences between the known total amount of Np added and the measured dissolved concentration.

Np removal by bentonite under anaerobic conditions with Na_2S. For comparison, Np removal by bentonite mediated by chemical reduction with Na_2S was also assessed, along with removal under aerobic conditions. The reactions were carried

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out in a bentonite suspension (0.5 g/L) also containing 0.01 mol/L $NaNO_3$ in 10-mL serum vials under an N_2 atmosphere. After boiling and cooling the suspension under O_2-free N_2 gas, the suspension was dispensed into vials, and then the vials were aluminum-capped with butyl rubber stoppers, and Na_2S solution was added to a final concentration of 0.01 mol/L. One hour later, the Np solution was added to the vials, at the indicated times, and pH and Eh (vs. NHE) were measured using a pH-ORP meter in a glove bag filled with O_2-free N_2, after which the suspensions were filtered through a 0.22 μm filter (Millipore), and the filtrate was analyzed as described above.

Np removal by microbial reactions. To assess the effect of *D. desulfuricans* on Np removal from bentonite suspension, microbial Np removal experiments were carried out in 10-mL glass serum vials containing 0.1 g/L bentonite suspended in 5 mL of cell cultivation medium. After sterilizing the vials, the cells were added and shaken in an incubator at 35 ˚C for 5 days. At that point, Np solution was added to the vials to a final concentration of 1×10^{-6} mol/L, and the vials were again shaken in the incubator under the same conditions used for cell cultivation. At the indicated times the pH, Eh, and Np concentration were analyzed as described above.

3. Results and Discussion

Effect of pH on Np removal by bentonite under aerobic conditions. The effect of pH on Np(V) removal by bentonite under aerobic conditions is shown in Figure 1. At pHs ranging from 1 to 10, there was no effect on Np adsorption while adsorption was enhanced at pH > 10. This result is in good agreement with previous researches.[10] With Np in aqueous solution under aerobic conditions, NpO_2^+ is the most stable and predominant species over a wide pH range;[11] indeed, it was present over nearly the entire pH range in the present experiment, which explains why pH did not affect adsorption at most of the pHs tested. In sum, this result shows that there is little or no adsorption of Np by bentonite under aerobic conditions. It would therefore seem inappropriate to use bentonite alone as an adsorbent to prevent Np(V) from migrating out of subsurface repositories into the environment even though it is a very effective adsorbent for other radionuclides.

Microbial and chemical removal of Np(V). Microbial removal of Np(V) from bentonite suspension was investigated as shown in Figure 2. For comparison, we also assessed Np removal mediated by chemical reaction under anaerobic condition with Na_2S (without cells), as well as under aerobic conditions. The pH and Eh (vs. NHE) conditions for this series were nearly constant throughout: under an aerobic condition, pH = 7.2 ± 0.2 and Eh = 333 ± 22 mV; with 0.01 mol/L Na_2S, pH = 7.2 ± 0.1 and Eh = –152 ± 20 mV; and with cells, pH = 7.8 ± 0.2 and Eh = –179 ± 14 mV. The removal of Np was achieved in the presence of *D. desulfuricans* and under anaerobic conditions with H_2S, whereas there was little or no removal under aerobic conditions. In the presence of cells, Np removal was most rapid, and the level of Np in the suspension was reduced by 95% or more within about 10 h, suggesting that *D. desulfuricans* reduces soluble Np(V) to insoluble Np(IV) enzymatically, although chemical reduction by H_2S produced by the cells cannot be ruled out. In addition, the reducing reagents (i.e. sodium ascorbate and thioglycolate) in the medium could affect the Np reduction as reported previously.[5] Moreover, the formation of low-solubility products of tetravalent actinide phosphate[12] should be investigated, as the medium contained phosphate for cell growth. Nonetheless, our findings suggest that sulfate reducing bacterium *D. desulfuricans* could remove Np from aqueous solution through direct and/or indirect microbial reactions and affect migration of Np in the subsurface environment.

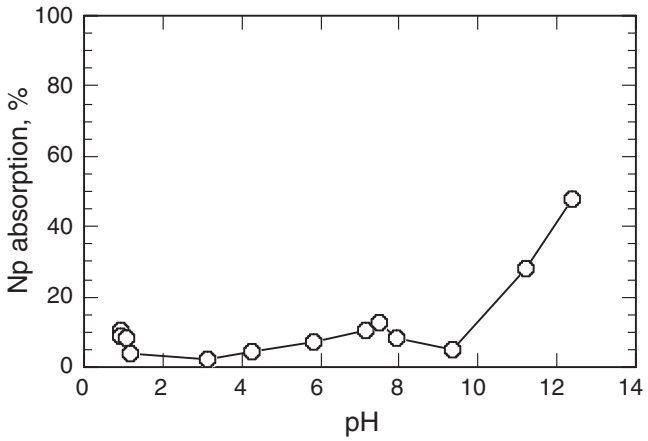

Figure 1. Adsorption of Np(V) on bentonite over a wide range of pHs under aerobic conditions.

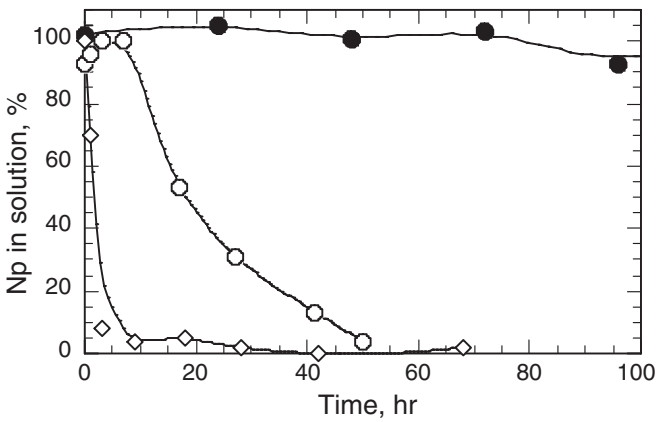

Figure 2. Microbial mediated removal of Np(V). Removal of Np by bentonite under aerobic conditions (●), with 0.01 mol/L Na_2S (○) and with cells (□). Conditions: bentonite, 0.5 g/L; $NaNO_3$, 0.01 mol/L; Np, 1×10^{-6} mol/L.

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