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THE INFLUENCE OF DESULFOVIBRIO DESULFURICANS ON NEPTUNIUM CHEMISTRY

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ABSTRACT

The role of biotic Np(V) reduction is studied in light of its potential role in the environmental immobilization of this hazardous radionuclide. The speciation of Np in *Desulfovibrio desulfuricans* cultures is compared with Np speciation in the spent medium and in the uninoculated medium. Precipitates formed in all three samples. Optical spectroscopy and x-ray absorption near edge structure (XANES) were used to determine that Np(V) is almost quantitatively reduced in all three samples and that the precipitate is an amorphous Np(IV) species. These results demonstrate that the reduction of Np is independent of *Desulfovibrio desulfuricans*. The underlying chemistry associated with these results is discussed.

INTRODUCTION

Neptunium (Np; atomic number 93) is a highly toxic, long-lived radionuclide that is abundant in nuclear waste. Like other light actinide elements, the chemistry of Np is complex and dependent upon speciation¹. Under environmentally relevant conditions, Np can be found in the (III), (IV), (V), or (VI) oxidation states. In the laboratory, acidic Np solutions at low pH contain primarily the neptunyl $[O=Np(V)=O]^+$ ion. Whereas Np(V) and (VI) are soluble in near neutral pH, Np(IV) forms hydrous oxides or the dioxide, both of which are very insoluble. Therefore, in order to effectively model the fate and transport of Np in the environment, it is important to understand to what extent neptunyl can be reduced to an insoluble Np(IV) species, and to identify any mechanisms by which such a reduction can occur.

Although the redox chemistry of Np is predictable under controlled, laboratory conditions, the complexity of natural groundwaters, in terms of dissolved organics and inorganics, as well as the presence of bacteria and catalytic mineral surfaces, prohibits a predictive understanding of Np chemistry and redox speciation in all but the simplest systems. The reduction of uranyl(VI) to UO_2 has been observed and understood in terms of an enzymatic reaction that is coupled to the electron-transport chains of metal-reducing bacteria^{2, 3}. The bacteria involved in the reduction are well known as anaerobic iron, Fe(III), reducers. Thermodynamically, the uranyl(VI) ion is harder to reduce than either the Np(V) ion or the Fe(III) ion, as evidenced by the standard reduction potentials listed in Table I.

A variety of microorganisms have been demonstrated to reduce uranium(VI)⁴ and Fe(III)⁵ under anaerobic conditions. The microbial influences on uranium and iron chemistries

suggest that Np speciation may be significantly altered by microbial growth. Significant microbial reduction of Np(V) would have a marked influence on the fate and transport of this radionuclide in the environment. Based on the literature reports cited above, and our previous experience with the growth of *Desulfovibrio desulfuricans*⁶, we have chosen to investigate the influence of this sulfate reducing anaerobic bacterium on Np chemistry.

Table I. The standard reduction potentials of some common metal ions⁷. The more positive the value, the more favored the reaction product.

Reduction Half-Cell	Potential (V)
$\text{UO}_2^{2+} + 4\text{H}^+ + 2\text{e}^- \rightarrow \text{U}^{4+} + 2\text{H}_2\text{O}$	+0.27
$\text{NpO}_2^+ + 4\text{H}^+ + \text{e}^- \rightarrow \text{Np}^{4+} + 2\text{H}_2\text{O}$	+0.66
$\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$	+0.771
$\text{Hg}^{2+} + 2\text{e}^- \rightarrow \text{Hg}^0$	+0.796

EXPERIMENTS

D. desulfuricans (ATCC 29577) were grown anaerobically at 30°C in Modified Starkey's Medium (ATCC 207) with lactate. Strict precautions were taken to ensure that Fe was excluded from the growth medium. In addition, Fe is known to facilitate the reduction of neptunyl(V). The solution pH was adjusted to 6.2 with hydroxide to stabilize pentavalent Np and to limit hydroxide formation while maintaining an environment suitable for bacterial growth. Neptunyl were added at the time of culture inoculation. Two control experiments were performed: (1) Np was added to uninoculated medium and (2) Np was added to spent medium. After incubation for 5 days in the presence of Np, motile cells were observed at 400x using an Olympus phase contrast microscope.

Optical data were obtained on an Olis-converted Cary-14 spectrophotometer. X-ray diffraction data were obtained on the filtered precipitates using a Scintag theta-theta diffractometer operating with a copper tube and a Peltier detector. The Np L₃-edge (17610 eV) x-ray absorption near edge structure (XANES) data were collected on 12BM-B, the BESSRC bending magnet beamline at the Advanced Photon Source (APS). The beam line is equipped with Si<111> crystals in a double-crystal configuration. Harmonic rejection was accomplished using a Pt mirror, set to reject energies higher than about 25 KeV. Harmonic rejection at these energies is necessary at the APS because of the relatively high flux of high-energy photons. The energy was calibrated by setting the inflection point of the first derivative from the Zr K edge to 17.998 KeV. All data were taken in the fluorescence mode, using a flow-type ion chamber detector (The EXAFS Co.), which was purged with xenon and used without slits or a scattering radiation filter. No time-dependent spectral changes were observed over multiple sample scans.

RESULTS AND DISCUSSION

The sample of interest and the two controls all showed a white precipitate that formed within the first day of incubation at 30°C. The precipitate made it difficult to quantify microbial growth, however observable motile microbial cells after 5 days of incubation suggests some microbial growth under our experimental conditions. The solutions were all filtered, after which the remaining solute and the precipitate were treated separately. An x-ray powder pattern of the precipitate revealed no diffraction lines. The precipitate appears amorphous at copper-radiation wavelengths.

Figure 1. Left: The optical spectra of standard Np solutions containing Np(III), Np(IV), Np(V) and Np(VI). Right: Optical spectra of the growth medium immediately after inoculation (top) shows the presence of Np(V). All solutions after incubation (bottom) continue to show evidence of only Np(V). The intensity of the signal is much reduced however, indication loss of solution Np.

Optical spectra of the solutions were obtained before and after incubation and filtration. The spectra obtained are compared with standard Np spectra in Figure 1. Optical spectroscopy is often used as a characterization tool for the Np oxidation state in solution because of the well separated signature spectrum available for each oxidation state⁸, as demonstrated in the Figure. The spectrum of the Np in the growth medium immediately after inoculation with bacteria is consistent with that expected for Np(V)O_2^+ . Whereas the spectrum taken on the same sample after incubation and filtration also shows the presence of Np(V)O_2^+ , the intensity of the signal is significantly reduced. There is no evidence of Np(IV) in solution, even though the molar absorptivity of tetravalent Np is rather high. This result is expected because the solubility of Np(IV) is low at the pH of the solution. In contrast, because of the broad spectral feature associated with Np(VI), the presence of moderate amounts of the neptunyl(VI) moiety cannot be ruled out based on these results alone. Assuming a linear relation between absorbance and concentration (Beer's Law), these data provide evidence that much of the Np(V) has been removed from solution. The Np remaining in solution is indistinguishable by optical spectroscopy from that of the original solution, appearing as Np(V).

XANES spectroscopy supports this finding. The spectra from the Np solutions obtained from both the active bacteria and the spent medium after filtration indicate the presence of very little Np. In contrast, the precipitates obtained from these samples, as well as the control to which no bacteria were added, all show evidence of significant amounts of Np. The XANES spectra obtained from the solid precipitates are compared with signature XANES spectra of Np in the tri-, tetra-, penta-, and hexavalent oxidation states. XANES is a single ion probe that is very sensitive to oxidation state and has successfully been used to characterize Np speciation⁹, both in solution and in the solid phase. In most cases, the edge energy, defined as the maximum in the first derivative, is seen to shift to higher energy with increasing valence. The situation is somewhat more complex for U¹⁰, Np⁹ and Pu¹¹, all of which form the dioxo cations [O=An=O]ⁿ⁺. In these cases, the edge energy does not increase with increasing formal charge on the actinide ion so that any analysis of valence from XANES data must include the edge features to higher energy. This is demonstrated by the Np data from the standard solutions shown in Figure 2. The Np(IV) and Np(V)O₂⁺ spectra are similar, and are differentiated in part by the broad peak to higher energy observed for the latter species.

The data obtained from the three solutions are statistically indistinguishable and are represented in Figure 2 by the data from the Np in the spent medium. The edge energies and the peak shapes from these data are consistent with Np(V)O₂⁺ in solution. These results directly support the optical data from the same solutions. The data obtained from the solid precipitates are also indistinguishable among themselves. However, they are markedly different from the data obtained from the solutions. The data from all the solid precipitates are consistent with Np(IV).

Figure 2. Left: A comparison of the XANES obtained from Np in 4 different oxidation states. It can be seen from the figure that Np(IV) and Np(V) have similar edge positions. Right: A comparison of the XANES spectra obtained from the solution after incubation (dotted line) with that of the solid precipitate (solid line). These XANES data show that Np has been reduced in solution.

The XANES data clearly demonstrate the presence of Np(V) in solution and Np(IV) in the solid precipitates. The data from the sample in which Np was added to the culture medium with viable *D. desulfuricans* are indistinguishable from those in which Np was added to spent medium and from those in which Np was added to fresh medium without any *D. desulfuricans*. The precipitates formed in all samples at approximately the same rate during incubation. These results show that the reduction of Np(V) to Np(IV) was independent of the presence of *D. desulfuricans* on the timescale of days. Np(V) is rapidly and almost quantitatively reduced in the presence of the medium alone at 30°C.

The underlying assumption for the experimental protocol chosen herein was that NpO_2^+ would be stable in the culture medium and that the reduction of Np(V) would arise primarily from a biotic process, or as a secondary result of a biotic process. This assumption was based in part on the reduction potential listed in Table I for Np(V). This standard reduction potential represents the thermodynamic value obtained with Np in an acidic solution (pH=0) of perchloric acid. The culture medium required to grow *D. desulfuricans* differs markedly from this standard solution, and as a result the redox couple is expected to shift to lower potential¹². Furthermore, the neptunyl(V)/neptunium(IV) couple is not rapidly reversible in perchloric acid,¹³ however the kinetics can be significantly altered by the presence of other ions, such as iron. Perhaps the most important to consider are disproportionation reactions of the form:

Note that this equilibrium reaction is dependent on acid concentration in solution. In the presence of a complexing anion, the equilibrium will be shifted. For example, studies have shown that the equilibrium constant for this reaction is 4×10^{-7} in 1M perchloric acid, but is shifted to 2.4×10^{-2} in 1M sulfuric acid¹⁴. This shift in equilibrium results in a 10,000 fold increase in the concentration of Np^{4+} , which is insoluble and drops out of solution. Unfortunately, sulfate is an essential component of the medium because *D. desulfuricans* is a sulfate reducing bacterium, that is it derives its energy from the reduction of SO_4^{2-} . Np^{4+} hydrolyses readily at the pH of these experiments, forming an insoluble hydroxide according to¹:

Finally, it has been previously demonstrated that uranyl is reduced to U^{4+} oxides or hydroxides under hydrothermal treatment in the presence of an organic monolayer^{15, 16}. Iron(III) has been shown to reduce under similar conditions¹⁷.

Our results, supported by literature precedent, reveal that neptunyl(V) is reduced abiotically under the employed experimental conditions. We have not ruled out some concomitant biotic reduction of neptunyl, however the abiotic pathway is clearly dominant. Attempts to conduct biotic Np redox experiments in a simplified growth medium may provide further insight into the mechanism of Np reduction, but they are unlikely to provide insight into the fate of environmental Np in the presence of *D. desulfuricans*. The experiments discussed herein have demonstrated that the abiotic chemistry is controlling the Np speciation in conditions conducive to *D. desulfuricans* growth.

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