Biodegradation **13:** 329–342, 2002.

Reduction of Np(V) and precipitation of Np(IV) by an anaerobic microbial consortium

Bruce E. Rittmann[1,*], James E. Banaszak[1,2] & Donald T. Reed[3]
[1]*Department of Civil and Environmental Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3109, USA;* [2]*ExponentFailure Analysis Assoc., Two North Riverside Plaza, Suite 1400, Chicago, IL 60606, USA;* [3]*24700 W. Easy St., Plainfield, IL 60544, USA (*Author for correspondence)*

Accepted 15 November 2002

Key words: anaerobic consortium, bioreduction, complexation, hydrogen, modeling, Neptunium, precipitation, succinate

Abstract

A combination of experimental, analytical, and modeling investigations shows that an anaerobic, sulfate-reducing consortium reduced Np(V) to Np(IV), with subsequent precipitation of a Np(IV) solid. Precipitation of Np(IV) during growth on pyruvate occurred before sulfate reduction began. H_2 stimulated precipitation of Np(IV) when added alone to growing cells, but it slowed precipitation when added along with pyruvate. Increasing concentrations of pyruvate also retarded precipitation. Accumulation of an intermediate pyruvate-fermentation product – probably succinate – played a key role in retarding Np(IV) precipitation by complexing the Np(IV). Hydrogen appears to have two roles in controlling Np precipitation: donating electrons for Np(V) reduction and modulating intermediate levels. That Np(V) is microbially reduced and subsequently precipitated under anaerobic conditions has likely beneficial implications for the containment of Np on lands contaminated by radionuclides, but complexation by fermentation intermediates can prevent immobilization by precipitation.

Introduction

The application of biotechnology for the remediation of metal- and radionuclide- contaminated waste streams and groundwaters is the subject of much research and practical interest (Bachofen 1990; Francis 1994; Francis & Dodge 1993, 1994; Hinchee et al. 1995; Lovley 1995; Lovley & Woodward 1996; Mackaskie et al. 1997; Reed et al. 1991; Banaszak et al., 1998b; NRC 2000a, b). A major driving force behind much of this work is the need to cleanup contaminated sites at former nuclear-weapons facilities (Broido 1995). Past practices at many of these sites have resulted in the release of mixtures of radionuclides and other organic and inorganic wastes into adjacent soil and groundwater (Riley et al. 1992; NRC 2000b).

In contrast to biological treatment of organic contaminants, which often can be mineralized to harmless products, the treatment of metals and radionuclides cannot "destroy" the harmful material. Thus, the treatment goal for in situ bioremediation usually is the immobilization of the metal or radionuclide so that its migration is prevented (NRC 2000a). Similar approaches have been attempted or envisioned for treatment of waters or soils contaminated with radionuclides (Mackasie et al. 1994, 1995, 1996, 1997; Thomas & Mackaskie 1996; Yong & Mackasie 1995; Francis & Dodge 1994; Dodge & Francis 1994; Phillips et al. 1995).

Banaszak et al. (1999a) provide a comprehensive review of how microorganisms directly or indirectly affect the mobility of actinides (Plutonium, Pu; Uranium, U; Neptunium, Np; and Americium, Am). In brief, some microorganisms have a direct effect on actinides by using them as electron acceptors. In most cases, the reduced form is less mobile, because it precipitates or sorbs more strongly than does the oxidized form. Other microorganisms indirectly affect actinide mobility when they biodegrade complexing ligands that often are present as co-contaminants with

the actinides. Release of the actinide from the ligand usually makes it more susceptible to precipitation or sorption.

In this study, we investigate how an anaerobic microbial consortium brings about the precipitation of Np originally present in the relatively soluble +5 oxidation state. The documentation of microbially catalyzed Np precipitation is an important finding that adds to growing evidence that actinides can be immobilized by microbial means if the proper conditions are present (Banaszak et al. 1999a). It is particularly important because Np(V) is difficult to reduce and is highly mobile under normal subsurface conditions. In this study, we combine anaerobic microcosm experiments with X-ray spectroscopy and biogeochemical modeling to unravel how different electron donors accelerate or decelerate Np precipitation. Ironically, we provide evidence that the microorganisms produce, under certain conditions, a complexant that retards Np(IV) precipitation.

The specific objectives of the study are to:

(1) document that a sulfate-reducing consortium reduces Np(V) to Np(IV), which then precipitates;
(2) elaborate the roles that different electron donors – particularly H_2 – play in reducing and precipitating Np;
(3) identify the way in which a fermentation intermediate that complexes strongly to Np(IV) can prevent it from precipitating.

Materials and methods

Culture origin and growth conditions

The microbial culture used for these studies was obtained from the Environmental Research Division at Argonne National Laboratory. The consortium was isolated previously from creek sediment (Boopathy & Manning 1996). Characterization of the anaerobic, sulfate-reducing consortium identified four *Desulfovibrio* species, including *D. desulfuricans* A, *D. desulfuricans* B, *D. gigas*, and a strain closely resembling *D. vulgaris* (Boopathy 1994; Boopathy & Manning 1996). No characterization was performed for other anaerobic, fermentative, or facultative organisms in the consortium.

For these studies, the consortium was grown at 25° C in sealed 70-ml serum bottles using standard anaerobic techniques. Growth medium contained (in g/L): KH_2PO_4, 0.4; K_2HPO_4, 0.2; NH_4Cl, 0.5; NaCl, 0.6; $MgCl_2$, 0.1; $CaCl_2$, 0.5; Na_2SO_4, 2.8; yeast extract, 0.2; and Na-pyruvate, 3.3. Resazurin (0.5 mg/l) was added as a redox indicator. The pH of the medium was adjusted to 6.4 with NaOH. The medium was sterilized through a 0.2-μm filter and dispensed into sealed, sterile serum bottles using a needle syringe. The bottles were purged for 20 min with a sterile, high purity nitrogen or N_2/CO_2 (72/28%) gas mixture, leading to a change in color from blue to pink due to Resazurin reduction. Due to the higher carbonic acid concentration in equilibrium with the N_2/CO_2 mixture, the final pH of the medium was 5.4 when this gas was used. Cultures were maintained by needle-syringe transfer of a 5% inoculum from actively growing cultures to fresh medium.

Batch experiments

Batch experiments were conducted in sealed 10- and 30-ml serum bottles at 25 °C. The bottle microcosms were sealed with rubber stoppers and sterilized by autoclaving. The experimental medium used varied by experiment. Some experiments used the growth medium described above. For other experiments, a non-growth medium containing only the phosphate, ammonium, Na, Mg, Ca, and yeast extract was prepared as described above, filter-sterilized (0.2 μm), and added to the serum bottles with a needle syringe. The medium was non-growth because the donor (pyruvate) and acceptor (SO_4^{2-}) were absent. After addition of either medium, the bottles were purged with sterile N_2 or N_2/CO_2 for 20 min. For experiments studying the effect of different electron donors or an acceptor, concentrated stock solutions (750 mM) of donors (Na-pyruvate, -formate, -acetate, or - lactate) and the acceptor (Na_2SO_4) were prepared, added to sealed, sterile serum bottles, and sterilized by autoclaving. The stock solutions were regularly purged with sterile, high-purity He gas to maintain anoxic conditions. Sterile hydrogen gas was added as an electron donor through a syringe needle to give 6 or 12.3 mmol H_2 per liter of medium. Electron donors and acceptors were transferred to the experimental bottles using sterile and anaerobic techniques. The pH was monitored by combination glass electrode (no Np) or pH paper (with Np). The typical culture pH during growth on pyruvate increased from 5.4 to 5.8–5.9 over the course of the experiment.

Experiments were initiated by sterile, needle-syringe transfer of a 5% inoculum from an actively growing culture or by inoculation of a washed cell

suspension. The cell suspensions were prepared by transferring the culture into an anaerobic chamber, where it was rinsed with sterile phosphate buffer (20 mM) or de-ionized water, concentrated by centrifugation (4000 rpm × 10 min) three times, and resuspended in a serum bottle containing buffer or water. The serum bottle was sealed with a rubber stopper and removed from the anaerobic chamber for use. When rinsed cell suspensions were used in experiments, a test bottle containing regular growth medium was always inoculated with the rinsed culture. The test bottle was monitored for growth to ensure that the rinsing process did not harm the cells.

Preparation of sterile, anoxic Np(V) stock

A stock solution of Np(V) was prepared by column separation (Biorad AG-50) of ^{237}Np from Pu and Pa using a concentrated HCl rinse. The Np was fumed to near dryness in concentrated perchloric acid and re-dissolved in 2 ml of 0.01 M HCl, resulting in a predominantly Np(VI) solution. This solution was again diluted with 15 ml of 0.01 M HCl and electrolytically reduced for 30 min (–1.0 volt potential; 35 mA current; 0.5 volt current breakpoint). The oxidation-state purity of the Np stock was determined from its Vis-NIR spectrum. A comparison of the Np(V) absorption at 980.2 nm [Np(V)] to the total Np concentration obtained from scintillation counting 10 μl of solution was performed to establish the Ci purity of the Neptunium for counting purposes. The oxidation-state purity of Np(V) was always greater than 95% and typically greater than 99%. The stock solution concentration was 2.0 mM. Approximately 8 ml of the Np(V) stock was sterilized by filtration (0.2 μm) and transferred by needle syringe into a sterile, 10-ml serum bottle with a rubber stopper. The stock Np(V) solution was regularly purged with sterile, high-purity Ar gas to maintain anoxic conditions. Aliquots of Np(V) were added to experimental bottles by sterile, anoxic needle-syringe transfer.

To monitor the long-term stability of Np(V) in anoxic growth medium, serum bottles containing fresh, anoxic medium and sterile Np(V) stock were transferred into a flow-through N_2 glove box dedicated to actinide work. The Np(V) stock was allowed to equilibrate with the N_2 atmosphere for several days. The stock was added to the growth medium via needle syringe. Periodically, the solution was agitated and sampled with needle syringe inside the glove box and then transferred into a quartz cuvette modified with a Rotaflo high-vacuum Teflon stopcock to provide a gas-tight seal. The sealed cuvette was bagged out from the glove box for subsequent spectroscopic analysis.

Sampling and analytical methods

Microcosms were sampled periodically using a needle syringe. Biomass growth was monitored by optical density. Approximately 1 ml of solution was transferred into a quartz cuvette, and the optical density of the solution at 600 nm was measured with either a Spec 20 (no-Np samples) or CARY 5E (Np samples) spectrophotometer. Pyruvate, acetate, formate, sulfate, and other metabolic intermediate concentrations were determined by ion chromatography . Aliquots of solution were added to 0.7 ml of 0.01 M NaOH in autosampler tubes. The samples were vortexed to ensure proper mixing and analyzed with a Dionex DX-500 ion chromatograph (CD20 detector, GP40 pump, AS-11 column). The eluent concentrations and flow rates varied depending on the compound of interest. The instrument was calibrated using freshly prepared standard solutions of known concentration. The detection limits were approximately 10^{-6} M.

Neptunium chemical speciation was established by Vis-NIR spectroscopy (Varian CARY 5E) as described above. Soluble neptunium concentration was determined by comparison of scintillation counts (Packard model 2500 TR liquid scintillation counter) from equal volumes of 0.2-μm filtered and unfiltered aliquots of solution. Bottles were sampled with a needle syringe. A portion of the sample was transferred to a weigh dish. The needle was removed from the syringe and replaced with a 0.2-μm syringe filter. The remainder of the solution was filtered into another weigh dish. In this way, the samples were filtered before being exposed to air, minimizing the potential for oxidation of any precipitates. Known volumes were drawn from the weigh dishes with a pipette and added to scintillation cocktail (Ultima Gold) for subsequent counting. The detection limt depended on the counting time. For the counting conditions we used, it was approximately 10^{-7} M.

X-ray absorption near-edge spectroscopy (XANES) analyses were performed on the MR-CAT undulator beamline at the Advanced Photon Source (APS) at Argonne National Laboratory. Samples were prepared in a nitrogen glove box by gravity filtration (6000 rpm) through a 10,000 nominal molecular weight centrifuge filter (Micro Separation, Inc.). Following centrifugation, the filter was encapsulated in polystyrene plastic.

After encapsulation, the samples were removed from the glove box and mounted for XANES analysis. Np standards of various oxidation states were prepared by recovering solids from the following syntheses: $Np(IV)F_4$ by addition of excess fluoride to Np(IV); $Np(V)NaCO_3$ by titration of Np(V) stock with 0.1 M sodium carbonate to pH 7; and Np(VI)-phosphate by addition of excess phosphate to Np(VI) stock and adjustment of pH to 8.

Results

Stability of Np(V) in anoxic growth medium

To investigate the potential for abiotic Np(V) reduction by compounds in the growth medium in the absence of oxygen, the stability of $\sim 10^{-4}$ M Np(V) in anoxic growth medium was monitored by Vis-NIR for several months. Over the course of several weeks, the absorption at 980.2 nm, the band corresponding to the aquo NpO_2^+ ion , decreased by approximately 15%, but there was no evidence for the formation of Np(IV) in solution. Because the amount of Np(V) added initially exceeded the solubility of Np(V) in Ca-phosphate solutions (Fahey 1986), the 15% loss of Np(V) was as a Np(V)-phosphate precipitate. Thus, Np(V) was not reduced to Np(IV) in the growth medium when the microbial consortium was absent.

Toxicity of Np(V) toward the consortium

Previous work by Banaszak et al. (1998a) showed that Np(V) was chemically toxic to *Chelatobacter heintzii* at aquo NpO_2^+ free ion concentrations greater than about 2.8×10^{-5} M and toward *D. vulgaris* when the aquo NpO_2^+ ion concentration was greater than 4.4×10^{-6} M. To investigate the potential toxicity of Np(V) toward the consortium used for these studies, microbial growth was monitored at 1×10^{-5} and 2.5×10^{-4} M Np. The amount of growth observed for the culture at an Np concentration of 1×10^{-5} M Np(V) was similar to that seen by Boopathy & Manning (1996) in previous studies in the absence of inhibitory compounds. However, 2.5×10^{-4} M Np(V) decreased the growth by approximately 80%. For this reason, 1×10^{-5} M was the target Np(V) concentration for subsequent experiments to avoid complications from toxicity. The target concentration had the added benefit of preventing Np(V)-phosphate precipitation.

Fate of Np with non-growing cells

We performed experiments to investigate the ability of non-growing cells to precipitate Np. Omitting pyruvate and sulfate from the growth medium created non-growth conditions. To avoid pyruvate carry-over, the inoculum for these experiments was harvested in the log phase of growth and rinsed according to the procedure described above. The experimental microcosms were prepared as described previously (Lovley et al. 1993) for studying U(VI) reduction by sulfate-reducing bacteria, except that 10^{-5} M Np(V) was used instead of U(VI). Rinsed cells showed normal growth in the full medium (i.e., with pyruvate and sulfate present). Under non-growing conditions, the cells did not precipitate any Np when no electron donor was added or when H_2 or formate was supplied as an electron donor. The experiments were repeated in sterile, high purity water and in a 20-mM phosphate buffer, and no Np precipitation took place in either case. Thus, non-growing cells did not precipitate Np.

Fate of Np with growing cells

In initial studies with growing cells, Np additions were made when the cells were in the log phase of growth, along with or prior to additional electron-donor substrates. Np was precipitated for a variety of cell-growth conditions, but never in abiotic controls. For example, Figure 1 shows the fate of Np when the consortium received pyruvate as the electron donor with no electron acceptor addition. The Np was added after about 1 day of growth. The initial pyruvate concentration was 10 mM, and an additional 11 mM of pyruvate was added after 13 days. The cells precipitated approximately 66% of the Np after 40 days, as compared to the un-inoculated control, where no precipitation was detected. Although Figure 1 gives little information about the rate of Np precipitation, it documents that Np was precipitated when sulfate was absent.

Precipitation of Np also was observed in growing systems when sulfate was present in solution. Table 1 summarizes the additions of inocula (day 0), Np (day 4), and different electron donors (days 0, 4, 29, and 35). Figure 2, which shows the Np-partitioning results, identifies two key trends. First, Np precipitation occurred earlier and to a greater extent when 37.5 or 45 mM pyruvate was added as an electron-donor substrate, compared to when 73.2 mM was added. Second, the addition of H_2 (12.3 mM) as an electron donor appeared to stimulate Np precipitation in

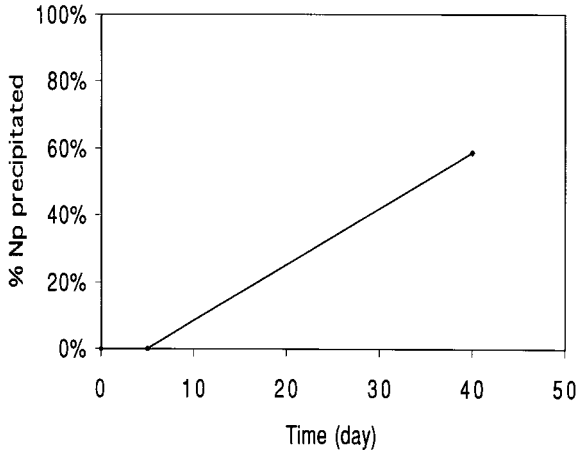

Figure 1. Loss of Np from solution in an anaerobic consortium growing on 10 mM pyruvate in the absence of sulfate. The Np was added after 1 day of growth. An additional 11 mM of pyruvate was added after 13 days. The line is used only to help visualize the results and does not imply a linear trend between 5 and 40 days.

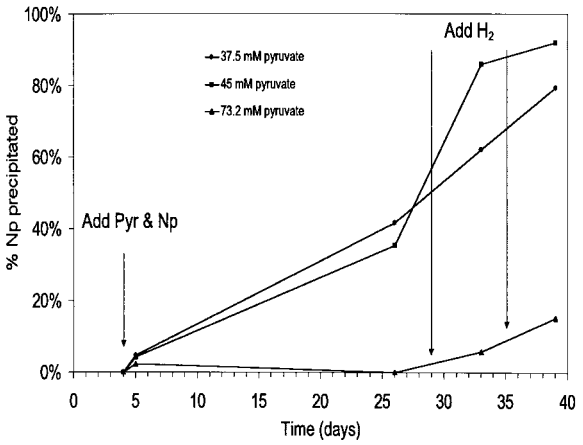

Figure 2. Fate of Np in the growth experiments in which 20 mM sulfate was present. Np precipitation was inhibited by the highest total pyruvate addition. The addition of hydrogen appeared to stimulate Np precipitation.

all cases. The precipitated Np was strongly associated with the biomass in these experiments, as the re-suspension of the biomass in 1 mM NTA solution did not re-solubilize the neptunium.

Additional experiments were performed to further investigate the role of H_2 in Np precipitation. Np(V) and electron-donor additions were made to cultures grown on 30 mM pyruvate and 20 mM sulfate for 4 days; the experimental set up is detailed in Table 2. Figure 3 shows the results from this experiment. Complete replacement of pyruvate with H_2 accelerated Np precipitation. However, addition of H_2 and pyruvate together slowed Np precipitation, as compared

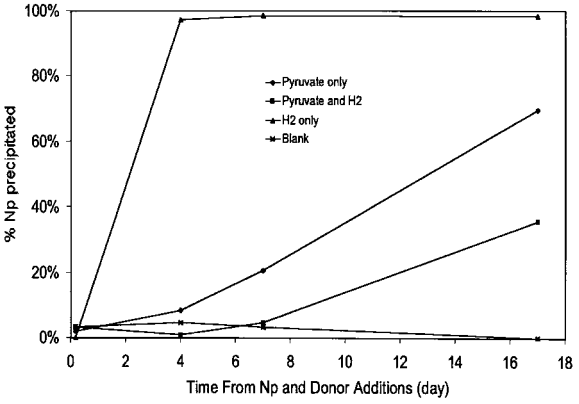

Figure 3. Fate of Np in growth experiments in which H_2 (12.3 mM) was or was not provided as an electron donor. Addition of hydrogen alone accelerated Np precipitation. Addition of hydrogen and pyruvate showed the slowest precipitation rate.

to adding either H_2 or pyruvate alone. The difference in precipitation rate was not caused by toxicity of Np, because the sample that showed the highest rate, which received only hydrogen as a supplemental electron donor, also had the highest Np concentration. At the end of the experiment (18 days), the pH of all samples was checked to verify that the precipitation was not caused by a pH-induced shift in Np chemical speciation. The pH of all bottles was from 5.9 to 6.1. Thus, the chemical speciation was not affected significantly.

Formate showed a stimulatory role toward Np precipitation similar to H_2. Figure 4 shows Np precipitation in cultures grown on pyruvate (30 mM). At time = 0, Np(V) was added to the cultures, which also were supplemented with formate (30 mM), formate (30 mM) and H_2 (6 mM), or pyruvate (30 mM). The precipitation results were nearly identical for the first two supplements. After 13 days, about 70% of the Np precipitated from solution, as compared to the 10–20% precipitation observed when only pyruvate was added. These results suggest that formate also was active in promoting Np precipitation.

Metabolism of the anaerobic consortium

Growth studies by Boopathy & Manning (1996) showed that the microbial consortium used in these studies achieved maximum growth on 15–30 mM pyruvate and 10–15 mM sulfate after approximately 15–20 days, and sulfide was detected in the cultures only after 20 days. We verified that the consortium carried out sulfate reduction and hydrogen consumption, but sulfate reduction (detected by the formation of sulfide and consumption of sulfate) began only after at least

Table 1. Summary of electron donor additions for growth experiment in which sulfate was supplied at 20 mM

Time of addition (days)	Sample No. 1	2	3
0	Inoculation + 30 mM Pyr.	Inoculation + 37.5 mM Pyr.	Inoculation + 65.7 mM Pyr.
4	Np + 7.5 mM Pyr.	Np + 7.5 mM Pyr	Np + 7.5 mM Pyr.
29	12.3 mM H_2	12.3 mM H_2	12.3 mM H_2
35	12.3 mM H_2	12.3 mM H_2	12.3 mM H_2

Table 2. Electron donor additions for growth experiments in which sulfate was supplied at 20 mM

Time of addition (days)	Sample No. 1	2	3	Blank
–4	Inoculation 30 mM Pyr.	Inoculation 30 mM Pyr.	Inoculation 30 mM Pyr.	N/A
0	Np + 15 mM Pyr.	Np + 15 mM Pyr. + 12.3 mM H_2	Np + 12.3 mM H_2	Np

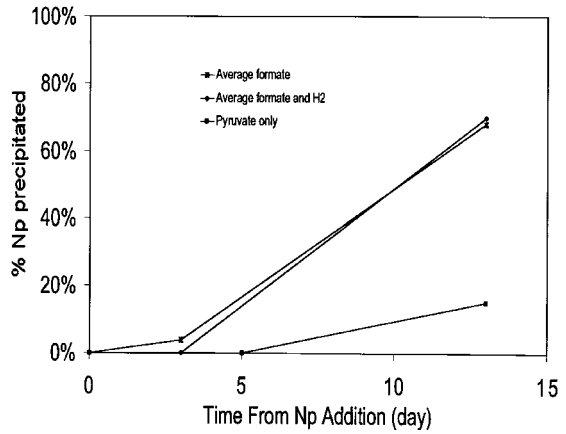

Figure 4. Comparison of Np precipitation by cultures grown on pyruvate and then supplemented with formate, formate + H_2, or pyruvate alone at time = 0. Np(V) also was added at time = 0. Formate appeared to show the same stimulatory role toward Np precipitation.

two weeks of incubation. The results of Np precipitation experiments clearly showed that, under certain conditions, the consortium catalyzed precipitation of the actinide within 7–8 days (particularly as shown in Figures 3 and 4), well before sulfide production was detected. These results indicate that the consortium was not carrying out sulfate respiration when much of the Np precipitation occurred. This implies that pyruvate was only being fermented during the time of Np reduction and precipitation, an issue explored more in the next series of experiments.

We conducted a series of growth experiments to investigate the metabolism of the consortium during the first week of growth on pyruvate. Figure 5 shows the concentration of the major substrates and intermediates, as well as and culture optical density for the first 190 h after inoculation of medium initially containing 30 mM pyruvate and 20 mM sulfate. Sulfate was not utilized during the course of the experiment (not shown in the figure). Pyruvate was completely utilized within the first 100 h of the experiment. Growth increased in parallel with pyruvate loss. Acetate, formate, and propionate accumulated during pyruvate degradation. The combined concentrations of acetate and propionate (the IC peak areas for these two compounds overlapped, even at the low eluent concentrations used for analysis) reached a maximum between 100 and 120 h, then showed a decreasing trend. After about 100 h, growth leveled off, although formate production continued until the end of the experiment. Furthermore, low concentrations (<1 mM) of succinate were detected transiently during the experiment; it reached its maximum concentration (0.88 mM) at about 144 h.

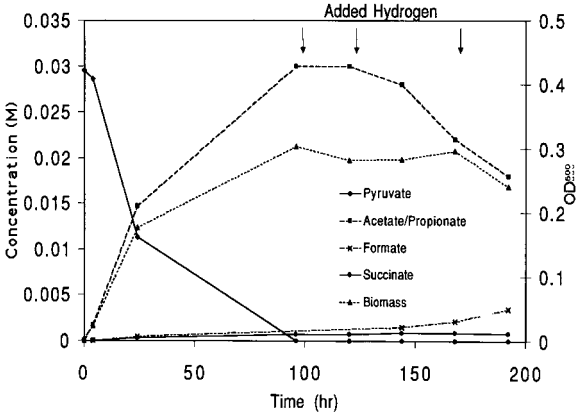

Figure 5. Concentration of major metabolites and intermediates during pyruvate degradation by the anaerobic consortium in the presence of 20 mM sulfate, which was not removed (not shown). Several fermentation products were formed, including succinate, which was detected at low (< mM) concentrations.

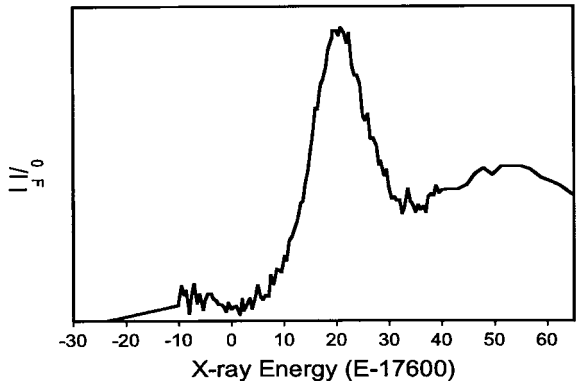

Figure 6. XANES spectra of precipitated Np from a growth experiment whose results are in Figure 2. The lack of the XANES shoulder at 25 eV associated with the linear dioxo structure of Np(V), the lack of a peak at just over 50 eV associated with Np(VI), and the peaks at 20 and 55 ev (characteristic of Np(IV) indicate that the precipitate contained mainly Np(IV).

Hydrogen additions (12.3 mM) at 98, 124, and 168 h failed to stimulate sulfate reduction. The persistence of sulfate in the growth medium confirms that the metabolism of the consortium in the initial stages of growth was fermentation, not sulfate reduction. Therefore, the state of the growth experiments at 100 h, when Np was added (e.g. Figure 3), was that pyruvate degradation was complete, but sulfate reduction had not begun. Furthermore, several organic-acid fermentation products were present: acetate/propionate, formate, and succinate.

Np oxidation state

XANES was used to determine the oxidation state of Np associated with the consortium under in situ conditions. Figure 6 shows a XANES spectrum of precipitated Np from the growth experiments whose results are shown in Figure 2. Because of the relatively low ($\sim 10^{-5}$ M) concentration of Np in these experiments, the spectra obtained had considerable noise. However, comparison of this spectrum with spectra we also obtained with the solid standards (Banaszak et al. 1999b) suggests that the spectrum of precipitated Np qualitatively matches that of Np(IV) because of the lack of the XANES shoulder associated with the linear dioxo structures of Np(V) and Np(VI) (Combes et al. 1992; Banaszak et al. 1999a) and the presence of characteristic Np(IV) peaks at 20 and 55 eV (Banaszak et al. 1999b). The presence of Np(IV) in the solid phase supports the hypothesis that Np precipitated following reduction of Np(V) to Np(IV).

Discussion and modeling analysis

Role of hydrogen

The experimental results clearly show that supplementing the anaerobic consortium with hydrogen or formate during growth could stimulate Np precipitation. Additionally, analysis by XANES spectroscopy indicated that Np(IV) was the dominant oxidation state of solid-phase Np. One hypothesis that explains the role of hydrogen (or formate as a hydrogen surrogate) in stimulating Np precipitation is that microorganisms that reduce Np(V) to Np(IV) require it at their electron donor. According to this hypothesis, H_2 is essential for the reduction of Np(V) to Np(IV), which can precipitate from solution.

Evidence for Np precipitation in systems receiving only pyruvate does not rule out hydrogen (or formate) as an essential donor for Np reduction, because pyruvate can be fermented to acetate and hydrogen in a reaction that provides sufficient free energy for growth regardless of the hydrogen concentration (Fauque et al. 1991; Hanson 1993; Peck 1993). A wide range of facultative and anaerobic microorganisms, including *Desulfovibrio* spp., can catalyze this reaction (Fauque et al. 1991; Hanson 1993; Peck 1993).

Understanding the role of hydrogen is complicated by the fact that propionate was detected in our studies (Figure 5) and in previous work with the consortium (Boopathy & Manning 1996). Figure 7 shows the alternative pathways of pyruvate fermentation to acetate, which produces H_2, and propionate, which consumes H_2. Succinate, also detected in our studies (Figure

5), is a key intermediate in the propionate pathway. As shown in Figure 7, high H_2 partial pressure shifts the fermentation-product distribution toward propionate (and succinate), but low H_2 partial pressure favors acetate fermentation. Secondary fermentation of propionate to acetate releases H_2.

For the conditions of growth experiments whose results are in Figure 3, the total quantity of H_2 added for the "H_2 only" microcosm was 3×10^{-4} mol. The maximum H_2 generated in the "pyruvate only" microcosm was 3.2×10^{-4} mol, assuming that all pyruvate ultimately was fermented to acetate. For the "H_2 plus pyruvate" microcosm, the maximum H_2 generated was 4.2×10^{-4} mol. This accounting shows that sufficient H_2 was available to drive Np reduction, whether or not H_2 was added.

The results from the entire set of growth experiments (Figures 2–5) provide evidence that Np(V) reduction and Np(IV) precipitation took place in all growth conditions, even when pyruvate was the only added electron donor and whether or not sulfate was present. When hydrogen (or formate) was added *after* initial growth on pyruvate, Np precipitation was stimulated. However, when H_2 was added along with pyruvate, Np precipitation was retarded. Since ample H_2 was available in all microcosms and H_2 sometimes retarded Np precipitation, another mechanism must be acting in addition to Np being reduced by H_2-oxidizing microorganisms.

Role of chemical speciation

Evidence that Np precipitation was slower when pyruvate (or pyruvate and hydrogen) was added concurrently with the actinide suggests that, after Np reduction occurred, Np(IV) precipitation depended on chemical speciation. In other words, reduction of Np(V) to Np(IV) was not sufficient to ensure Np precipitation. The most likely cause for slow Np(IV) precipitation was that it was complexed strongly to a ligand present in solution, because Np(IV) readily forms hydroxide complexes that lead to oxide and oxy-hydroxide precipitates (Rai et al. 1987, 1998).

The rapid biodegradation of pyruvate (Figure 5) indicates that complexation of Np(IV) by pyruvate was not the reason that precipitation of Np(IV) was slowest when H_2 and pyruvate were added together, even though it was fastest when H_2 was added alone. On the other hand, strong complexation of Np(IV) with a product of pyruvate fermentation is a likely explanation. Several observations from the experiments qualitatively support this hypothesis.

(1) Acetate, propionate, and succinate accumulated during the initial stages of pyruvate fermentation (Figure 5).
(2) The consortium only reached about 60% of its maximum growth during the first 4–8 days; peak growth occurred after 2 weeks, presumably due to utilization of fermentation products.
(3) Np precipitation was slowest in the samples receiving the largest supplemental pyruvate additions (Figure 2), presumably when the greatest accumulation of fermentation products took place.

Acetate and propionate, which have similar and low reported formation constants with actinides (Banaszak et al. 1999a; Mikhailov 1973), form complexes too weak to prevent Np(IV) precipitation. This is supported by the observation that more than 90% of 10^{-5} M Pu(IV) precipitated from a pH 5.7, 50-mM acetate solution (Weigel et al. 1986). Formate is a weaker ligand for Np(IV) than are either sulfate or acetate (Mikhailov 1973); therefore, it can be ruled out as well. Remaining potential ligands for Np(IV) complexation are carbonate, succinate, or unknown pyruvate products.

Carbonate is a known strong ligand for actinides in all oxidation states. In our system, pyruvate mineralization increased the total carbonate (headspace + liquid) by 50–100%. However, the pH was in the range of 5–6. Equilibrium-speciation modeling indicates that doubling the total carbonate in the system has very little effect on the calculated Np(IV) solubility in this pH range (Banaszak 1999). These modeling results are supported by experimental observations that show carbonate does not have a significant effect on Pu(IV) and Np(VI) solubility until the pH enters the alkaline regime, usually above pH 8 (Capdevila et al. 1996; Eiswirth et al. 1985; Kim et al. 1983; Nitsche & Silva 1996; Pratopo et al. 1990; Banaszak et al. 1998b; Yamaguchi et al. 1994). Finally, Pratopo et al. (1990) and Efurd et al. (1998) concluded that $Np(OH)_{4(am)}$ controlled Np(IV) solubility in high-carbonate systems, even at alkaline pH.

We hypothesize that Np(IV) precipitation was affected by the accumulation of an intermediate fermentation product having strong complexing capability during the initial stages of fermentation by the anaerobic consortium. Because succinate was detected at a significant concentration that persisted (Figure 5) and should complex strongly with Np(IV) (dis-

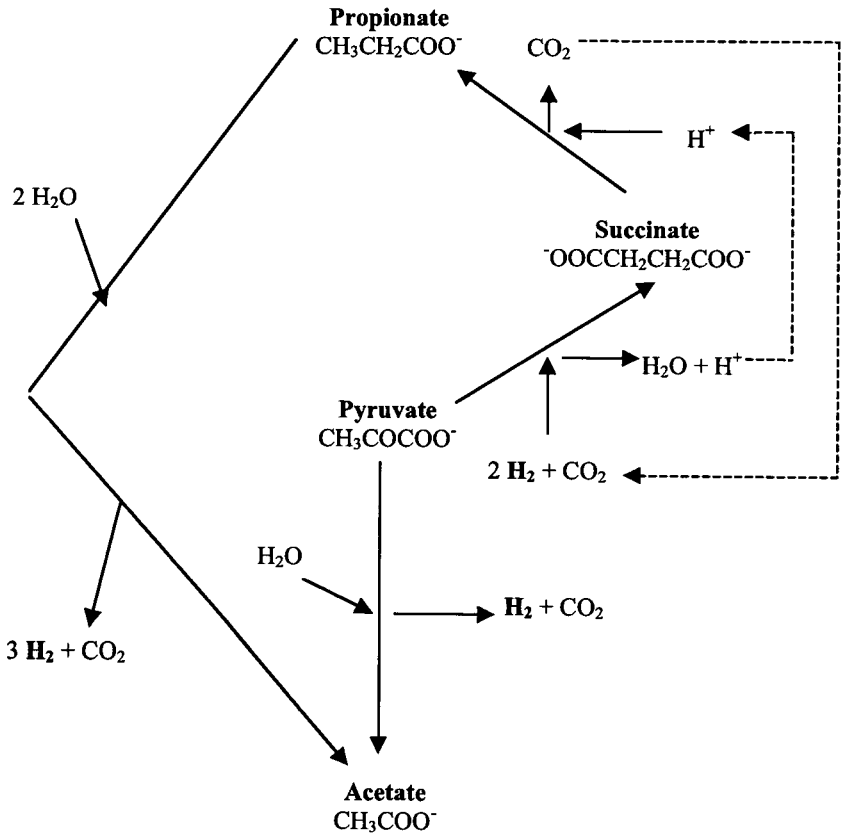

Figure 7. Alternate pathways of propionate and acetate formation from pyruvate fermentation by *Desulfobulbus propionicus*. A key intermediate in the propionate pathway is succinate. The H^+ and CO_2 cycling shown on the diagram (dashed arrows) represent internal transcarboxylation of propionyl-CoA to methylmalonyl-CoA. During pyruvate fermentation, high hydrogen partial pressures favor propionate formation. High hydrogen partial pressure inhibits fermentations leading to acetate. Adapted from Hansen (1993) and Madigan et al. (2000).

cussed below), we think that it is the most likely intermediate. However, an unknown intermediate cannot be ruled out. With this scenario, precipitation of Np(IV) depends on the degradation of succinate, which otherwise forms a strong complex that keeps Np(IV) in solution. Several key observations qualitatively support this scenario. First, Np precipitation was retarded by larger pyruvate additions. If the pyruvate-fermentation stoichiometry did not change significantly, larger pyruvate additions should result in higher succinate concentrations. Second, when pyruvate was added with hydrogen, Np precipitation was retarded. This observation is consistent with the shifting of fermentation reactions toward reduced-products (i.e., propionate via succinate) when hydrogen partial pressures are high (Figure 7). Finally, when only hydrogen was added as a supplemental electron donor, Np precipitation proceeded rapidly, because succinate could not be generated without pyruvate fermentation.

In addition to the evidence presented in this work, the proposed explanation for control of Np precipitation is supported by recent mathematical and experimental studies on the solubilization and mobilization of actinides by organic degradation products at acidic pH. In the Np(IV) system, Rai et al. (1998) showed that isosaccharinic acid (ISA), a degradation product of cellulose, significantly increased the solubility of Np(IV). For example, the measured Np solubility at pH 6 in 8 mM ISA was approximately 10^{-5} M, many orders of magnitude higher than the predicted solubility of Np(OH)4(am), and was attributed to the formation of mixed hydroxide-ISA complexes (Rai et al. 1998). In column studies of U(VI) transport in pH 5-6 groundwater, Read et al. (1998) found that saccharic acid was more effective than EDTA in removing pre-adsorbed U(VI) from the column. Modeling calculations by these researchers suggested that U speciation was dominated by complexation with saccharic acid in this pH regime. In a modeling study of Pu(IV)

fate at a disposal site, Stockman (1998) showed that Pu speciation was initially dominated by complexation with organic degradation products. According to the model simulations, subsequent biodegradation of these compounds caused a shift in Pu complexation to carbonate.

It might be argued that a reduced complexant, such as succinate, would complex Np(V) and then reduce it to Np(IV). This scenario is very unlikely, because Reed et al. (1998) showed that reduction of Np(V) by organic chelating agents proceeds very slowly and does not contribute to the net reduction of Np(V) in experimental systems like ours.

Modeling analysis of the effect of succinate

We utilized the biogeochemical model CCBATCH to evaluate the feasibility of our hypothesis that accumulation of succinic acid prevented the precipitation of Np(IV). The original CCBATCH model (VanBriesen & Rittmann 1999, 2000; Rittmann & VanBriesen 1996) couples microbially catalyzed reactions, which are kinetically controlled, with aqueous-phase acid/base and complexation reactions, which are at thermodynamic equilibrium. Rittmann et al. (2002) added a sub-model that links kinetically or equilibrium-controlled precipitation/dissolution to the microbial and aqueous-phase reactions. CCBATCH was designed to describe batch reactions, such as those performed in this study. The microbial sub-model includes oxidation of an electron-donor substrate (e.g., pyruvate in our studies), formation and utilization of intermediates (e.g., succinate), synthesis and endogenous decay of biomass, stoichiometric utilization of an electron acceptor, and stoichiometric consumption or generation of inorganic carbon, ammonium-nitrogen, and acidic hydrogen. The kinetic feature of the precipitation/dissolution sub-model predicts the precipitation or dissolution rate based on a "difference from equilibrium" approach that explicitly considers the concentration of the rate-limiting cation or anion and an intrinsic rate coefficient based on specific surface area and the rate-controlling mechanism. The equilibrium feature of the sub-model precipitates or dissolves just the amount of solid phase so that the aqueous phase speciation of the cation and anion match the solubility product (K_{sp}) for every time step. In general, precipitation consumes basic species, and the sub-model represents this through stoichiometric production of acidic hydrogen. Finally, CCBATCH predicts pH changes based on changes in acidic hydrogen and solving a proton condition.

To use CCBATCH, we needed sets of coefficients to represent the microbial reactions; the acid/base, complexation, and precipitation reactions of Np(IV) with common components of the medium; and the complexation of succinate with Np(IV). Table 3 summarizes the parameters used to describe pyruvate biodegradation and biomass growth on it: $q_{pyruvate}$ = maximum specific rate of pyruvate utilization, $K_{s,pyruvate}$ = half-maximum-rate concentration for pyruvate, $Y_{pyruvate}$ = true yield of biomass from pyruvate fermentation to acetate and propionate, and b = first-order endogenous-decay rate. The yield and stoichiometry for all components involved in pyruvate (CH_3COCOO^-) fermentation were based on the following overall reaction for fermentation to acetate (CH_3COO^-) and succinate ($(CH_2COO^-)_2$) coupled to biomass synthesis (McCarty 1972, Rittmann & McCarty 2001):

$$\begin{aligned} CH_3COCOO^- \; &+ \; 1.2305\, H_2O + 0.0635\, NH_4^+ \\ &\rightarrow \; 0.0635\, C_5H_7O_2N + 0.0655 \\ &\times \; (CH_2COO^-)_2 + 0.855 \\ &\times \; CH_3COO^- + 0.7105\, H_2CO_3 \\ &+ \; 0.471 H_2 + 0.0495\, H^+. \end{aligned}$$

Although some SRB are capable of utilizing succinate, the growth yields and substrate degradation rates are very low (Hanson 1993). Thus, we assumed that only 10% of the cells generated during pyruvate degradation were capable of succinate utilization, that no cells grew on succinate, and that the maximum specific rate of succinate utilization (based on the concentration of $HSuc^-$) was 5% that of pyruvate.

Table 4 lists the formation constants for the major aqueous-phase species of Np(IV) included in the modeling. The last entry in Table 4 is the solubility product for $Np(OH)_{4(am)}$, the critical solid phase for Np(IV). Np(IV) precipitation was modeled with the equilibrium feature of the precipitation/dissolution sub-model in CCBATCH (Rittmann et al. 2002).

The presence of two carboxylate groups at the ends of the succinate molecule, $^-OOC\text{-}CH_2\text{-}CH_2\text{-}COO^-$, which is functionally similar to oxalate, suggests that succinate may be a strong ligand for actinide complexation. Although there are no reported formation constants for oxalate with Np(IV), qualitative evidence has shown that oxalate forms stable complexes with +4 actinides (Fahey 1986). Cleveland (1970) reported overall formation constants (in acidic solutions an

Table 3. Kinetic parameters for Pyr.uvate degradation

Parameter	Value	How estimated
$q_{Pyr.uvate}$	1.6 mole Pyr./mole cell-h	Cooney et al. (1996)
$K_{s,Pyr.uvate}$	0.04 μM for [HPyr.]	Best fit to data
	$\sim$50 μM for $C_{T,Pyr}$	in Figure 8
$Y_{Pyr.uvate}$	0.0635 mole	After McCarty (1972)
	cells/mole Pyr.	and Rittmann & McCarty (2001)
b	0.05/d	Rittmann & McCarty (2001)

Table 4. Formation constants for major Neptunium(IV) aqueous complexes at ionic strength = 0.1

Species	$\beta^1_{x(y)z}$	Ref.
$NpOH^{3+}$	$\beta_{1(-1)0} = 10^{-2.10}$	Clark et al. (1995)
$Np(OH)_2^{2+}$	$\beta_{1(-2)0} = 10^{-5.32}$	estimate
$Np(OH)_3^{+}$	$\beta_{1(-3)0} = 10^{-8.55}$	estimate
$Np(OH)_4^{0}$	$\beta_{1(-4)0} = 10^{-11.80}$	Pratopo et al. (1989); Yamamuchi et al. (1994)
$Np(OH)_5^{-}$	$\beta_{1(-5)0} = 10^{-23.70}$	Clark et al. (1995)
$NpCO_3^{2+}$	$\beta_{1(0)1} = 10^{12.30}$	Nitsche (1991)
$Np(CO_3)_2^{0}$	$\beta_{1(0)2} = 10^{23.35}$	Nitsche (1991)
$Np(CO_3)_3^{2-}$	$\beta_{1(0)3} = 10^{30.00}$	Nitsche (1991)
$Np(CO_3)_4^{4-}$	$\beta_{1(0)4} = 10^{33.00}$	Nitsche (1991)
$Np(CO_3)_5^{6-}$	$\beta_{1(0)5} = 10^{34.00}$	Nitsche (1991)
$NpLac^{3+}$	$\beta_{1(0)1} = 10^{4.64}$	Lundqvist et al. (1984) based on $Ulac^{3+}$
$NpAc^{3+}$	$\beta_{1(0)1} = 10^{5.31}$	Moskvin (1969)
$NpSO_4^{2+}$	$\beta_{1(0)1} = 10^{3.50}$	Jones & Choppin (1969)
$Np(OH)_{4(am)}$	$K_{sp} = 10^{-54.5}$	Rai et al. (1987)

[1]Where $\beta_{x(y)z} = [M_xH_yL_z]/[M]^x[H]^y[L]^z$ or $\beta_{x(y)z} = [M_x(OH)_yL_z][H]^y/[M]^x[L]^z$.

ionic strength of zero) for Pu(IV) with oxalate of 11.0, 20.3, 26.8, and 29.2 for log β_1, log β_2, log β_3, and log β_4, respectively. On the other hand, the recent work of Rai et al. (1998) suggests that, for Np(IV), mixed hydroxide-organic acid complexes may be more important at near-neutral pH. Rai et al. (1998) reported a log formation constant of 36.9 for the complex $Np(OH)_2(ISA)_2$. At pH 6, the hydroxide concentration is about 10^{-8} M, depending on ionic strength. Thus, the "effective" log formation constant for the complex at pH 6 is $36.9 - 16 = 20.9$, very similar to that for $PuOx_2$.

When succinate forms a strong complex with Np(IV), it is likely that complexation decreases its availability as a substrate, slowing the rate at which it can be degraded (VanBriesen & Rittmann 1999, 2000). Similar results have been reported for strong complexes formed with other +4 actinides. For example, NTA degradation by *C. heintzii* was slowed significantly when Pu(IV) was present, even when no inhibition was observed (Banaszak et al. 1998b; Reed et al. 1999). Assuming that the complexes formed between succinate and Np(IV) are similar in stability to the Np(IV)–ISA complexes reported by Rai et al. (1998), we use a formation constant of $10^{20.9}$ for $Np(Suc)_2$.

As a feasibility check that the biodegradation of succinate can control Np(IV) precipitation, we ran a CCBATCH modeling experiment for the 96-h results from the pyruvate-only microcosm of Figure 3. Recall that, in this experiment, 15 mM pyruvate was added with Np(V) after 4 days (96 h) to a culture grown on 30 mM pyruvate. To match these conditions, we set the initial succinate concentration to 0.88 mM, the initial pyruvate concentration to 15 mM, and the initial cell concentration to 2 mM. The cell and succinate concentrations were taken from the 96-h data in Figure 5, and the pyruvate concentration is the added amount. Furthermore, we assumed that 3% (0.88 mM/30 mM) of any pyruvate degraded by the consortium produced succinate as an intermediate.

Figure 8 shows that, based on the stated assumptions, complexation by succinate could significantly affect the fate of Np(IV) in the system. According to the model calculations, complexation with the initial succinate in solution and the additional succinate produced during degradation of the supplemental 15-mM pyruvate prevents Np(IV) precipitation for about 50 h. When no complexation between succinate and Np is allowed (by setting the formation constant to a very small value), model calculations indicate complete precipitation within this time. Furthermore, model calculations indicate that the rate of Np precipitation, once initiated, directly depends on the rate of succin-

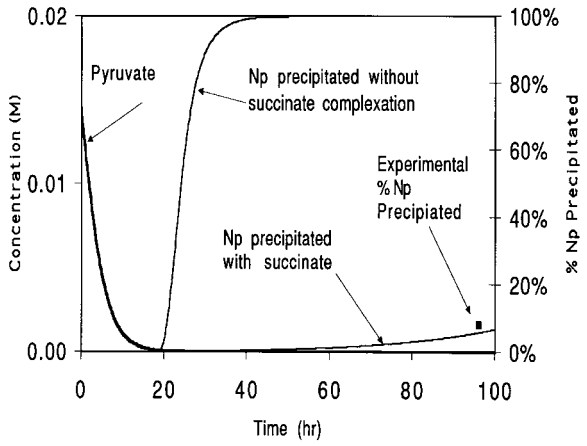

Figure 8. Model-calculated Np(IV) precipitation with and without Np(IV) complexation by succinate. Slow degradation of the intermediate could account for the delayed Np precipitation observed when pyruvate was added as a supplemental electron donor.

ate biodegradation. This modeling simulation supports the hypothesis that production and consumption of an organic fermentation product that strongly complexes Np(IV) controls its precipitation.

Implications and limitations

Our results support that H_2 is a key electron donor for Np(V) reduction to Np(IV), while accumulation of an organic intermediate with strong complexing properties can prevent or slow the precipitation of Np(IV). This finding implies that bioremediation designs based on supplementation of electron donor may work best when only the simplest electron donors, hydrogen and formate, are added.

Extrapolation of our results must be done cautiously, due to limitations in the work. We do not know which microorganisms in our consortium were responsible for Np(V) reduction or the release of succinate or, possibly, another intermediate. Still open are the stability constants of Np(IV) with various ligand, including succinate. We also do not know if the same trends would occur with other consortia or at a particular field site. Finally, the results highlight that more research is needed to determine the ability of fermentation intermediates, particularly succinate, to complex actinides.

Conclusions

We combined experimental, analytical, and modeling tools to show that an anaerobic, sulfate-reducing consortium reduced Np(V) to Np(IV), with subsequent precipitation of a Np(IV) solid. Precipitation of Np(IV) during growth on pyruvate did not require sulfate reduction, but occurred before sulfate reduction began. The addition of H_2 stimulated Np(IV) precipitation when added to growing cells, but it slowed precipitation when added along with more pyruvate. Higher concentrations of pyruvate also retarded precipitation.

The results suggest that formation of an intermediate compound – possibly succinate – during pyruvate fermentation played a key role in retarding Np(IV) precipitation by strongly complexing with the Np(IV). Thus, reduction of Np(V) to Np(IV) did not ensure precipitation; transformation of the intermediate also was required so that the Np(IV) was released from the soluble complex. H_2 accelerated Np precipitation in some cases, but retarded it in others. The acceleration was related to hydrogen's role as an electron donor for Np(V) reduction. On the other hand, H_2 retarded Np(IV) precipitation when it caused the build up of succinate, which complexes strongly with Np(IV) and prevents it from precipitating.

Acknowledgements

This work was supported in part by the Nuclear Energy Research Initiative (NERI) project 'Neptunium Speciation in Spent Fuel'. It also was supported in part by a grant from the Subsurface Science Program of the United States Department of Energy; the continued support of Dr. Frank Wobber is gratefully acknowledged. Work performed at MR-CAT is supported by funding from the Department of Energy under grant number DEFG0200ER45811. Use of the Advanced Photon Source was supported by the Department of Energy, Basic Energy Sciences, under contract no. W-31-109-Eng-38.

References

Artinger R, Marquardt CM, Kim JI, Siebert A, Trautmann N & Kratz JV (2000) Humic colloid-borne Np migration: influence of the oxidation state. Radiochimica Acta 88: 609–612

Bachofen R (1990) Microorganisms in nuclear waste disposal – Introduction. Experientia 46: 777–797

Banaszak JE (1999) Coupling microbial and mineral-phase reactions, Ph.D. dissertation, Dept. of Civil Engineering, Northwestern University, Evanston, IL.

Banaszak JE, Reed DT & Rittmann BE (1998a) Speciation-dependent toxicity of neptunium(V) towards Chelatobacter heintzii. Environ. Sci. Technol. 32: 1085–1091

Banaszak JE, VanBriesen JM, Rittmann BE & Reed DT (1998b) Mathematical modeling of the effects of aerobic and anaerobic chelate biodegradation on actinide speciation. Radiochimica Acta 82: 445–451

Banaszak JE, Reed DT & Rittmann BE (1999a) Subsurface interactions of actinide species and microorganisms: implications for the bioremediation of actinide-organic mixtures. J. Radioanalyt. Nucl. Chem. 241: 385–435

Banaszak JE, Webb SM, Rittmann BE, Gaillard J-F & Reed DT (1999b) Fate of neptunium in an anaerobic, methanogenic microcosm. In: Scientific Basis for Nuclear Waste Management XXII, November 30–December 4, 1998 (pp 1141–1149). Materials Research Society, Boston, MA

Bertsch PM, Hunter DB, Sutton SR, Bait S & Rivers ML (1994) In situ chemical speciation of uranium in soils and sediments by micro x-ray absorption spectroscopy. Environ. Sci. Technol. 28: 980–984

Boopathy R (1994) Anaerobic removal of TNT by sulfate-reducing bacteria. In: Emerging Technologies in Hazardous Waste Management, VI (p 56). American Chemical Society, Atlanta, GA

Boopathy R & Manning JF (1996) Characterization of partial anaerobic metabolic pathway for 2,4,6-trinitrotoluene degradation by a sulfate-reducing bacterial consortium. Can. J. Microbiol. 42: 1203–1208

Bourbon X & Toulhoat P (1996) Influence of organic degradation products on the solubilisation of radionuclides in intermediate and low level radioactive sastes. Radiochimica Acta 74: 315–319

Broido MS (1995) Natural and accelerated bioremediation research (NABIR). DOE/ER-0659T, US Department of Energy, Washington, DC

Capdevila H, Vitorge P, Giffaut E & Delmau L (1996) Spectrophotometric study of the dissociation of the Pu(IV) carbonate limiting complex. Radiochimica Acta 74: 93–98

Choppin GT. (1992) The role of natural organics in radionuclide migration in natural aquifer systems. Radiochimica Acta 58/59: 113–120

Clark DL, Hobart DE & Neu MP (1995) Actinide carbonate complexes and their importance in actinide environmental chemistry. Chem. Rev. 95: 25–48

Cleveland JM (1970) The Chemistry of Plutonium, Gordon and Breach Science Publishers, New York

Combes J-M, Chisholm-Brause CJ, Brown Jr GE, Parks GA, Conradson SD, Eller PG, Triay IR, Hobart DE & Meijer A (1992) EXAFS spectroscopic study of neptunium(V) sorption at the alpha-FeOOH/water interface. Environ. Sci. Technol. 26: 1376–382

Conradson SD (1998) Application of x-ray absorption fine structure spectroscopy to materials and environmental science. Appl. Spectro. 52(7): 252A–279A

Cooney MJ, Roschi E, Marison IW, Comninellis C & von Stockar U (1996) Physiological studies with the sulfate-reducing bacterium Desulfovibrio desulfuricans: evaluation for use in a biofuel cell. Enzyme Microb. Technol. 18: 358–365

Dodge CJ & Francis AJ (1994) Photodegradation of uranium-citrate complex with uranium recovery. Environ. Sci. Technol. 28: 1300–1306

Eiswirth M, Kim JI & Lierse C (1985) Optical absorption spectra of Pu(IV) in carbonate/bicarbonate media. Radiochimica Acta 38: 197–201

El-Naggar JA, Ess El-Din MR & Sheha RR (2000). Speciation of neptunium migration in under ground. J. Radioanalyt. Nucl. Chem. 246: 493–504

Fahey JA (1986) Neptunium In: Katz JJ, Seaborg GT & Morss LR (Eds) The Chemistry of the Actinide Elements, 1 (pp 443–498). Chapman and Hall, New York

Fauque G, Legall J & Barton LL (1991) Sulfate-reducing and sulfur-reducing bacteria. In: Shively JM & Barton LL (Eds) Variations in Autotrophic Life (pp 271–337). Academic Press, London

Francis AJ (1994) Microbial transformations of radioactive wastes and environmental restoration through bioremediation. J. Alloys Compounds 213/214: 226–231

Francis AJ & Dodge CJ (1993) Reclamation with Recovery of Radionuclides and Toxic Metals from Contaminated Materials, Soils, and Wastes. Technology 2002, The Third National Technology Transfer Conference and Exposition, Baltimore, MD, December 1–3, 1992, NASA

Francis AJ & Dodge CJ (1994) Waste Site Reclamation With Recovery of Radionuclides and Toxic Metals (p 65). US Associated Universities Inc, Oak Ridge, Tennessee

Francis AJ, Dodge CJ, Lu F, Halada GP & Clayton CR (1994) XPS and XANES studies of uranium reduction by *Clostridium* sp. Environ. Sci. Technol. 28: 636–639

Hanson TA (1993) Carbon metabolism of sulfate-reducing bacteria. In: Odom JM & Singleton Jr R (Eds). The Sulfate-Reducing Bacteria: Contemporary Perspectives (pp 21–40). Springer-Verlag, New York

Hayes KF & Katz LE (1996) Application of x-ray absorption spectroscopy for surface complexation modeling of metal ion sorption. In: Brady PV (Ed). Physics and Chemistry of Mineral Surfaces (pp 147–223). CRC Press, Boca Raton, FL

Hinchee RE, Means JL & Burris DR (Eds) (1995). Bioremediation of Inorganics. Batelle Press, Columbus, OH

Jones AD & Choppin GR (1969) Complexes of actinide ions in aqueous solution. Actinides Rev. 1: 311–366

Kim JI & Marquardt CM (1999). Chemical reaction of Np(V) with humic colloids in groundwater: influence of purification on the complexation behaviour, Radiochimica Acta 87: 105–108

Kim JI, Lierse C & Baumgartner F (1983) Complexation of the plutonium(IV) ion in carbonate-bicarbonate solutions. In: Carnall WT & Choppin GR (Eds). Plutonium Chemistry (p 319). American Chemical Society, Washington, DC

Kumata M & Vandergraaf TT (1998) Experimental study on neptunium migration under in situ geochemical conditions. J. Contam. Hydrol. 35: 31–40

Li Y, Kato Y & Yoshida Z (1993) Electrolytic preparation of neptunium species in concentrated carbonate media. Radiochimica Acta 60: 115–119

Lovley DR (1995) Bioremediation of organic and metal contaminants with dissimilatory metal reduction. J. Indust. Microbiol. 14: 85–93

Lovley DR & Woodward JC (1996) Mechanisms for chelator stimulation of microbial Fe(III)-oxide reduction Chem. Geol. 132: 19–24

Lovley DR, Roden EE, Phillips EJP & Woodward JC (1993) Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Marine Geol. 113: 41–53

Lundqvist R, Lu J-F & Svantesson I (1984) Hydrophillic complexes of the actinides. III. Lactates of Am^{3+}, Eu^{3+}, U^{4+}, and UO_2^{2+}. Acta Chemica Scandinavica A 38: 501–512

Macaskie LE, Jeong BC & Tolley MR (1994) Enzymically accelerated biomineralization of heavy metals: application to the removal of americium and plutonium from aqueous flows. FEMS Microbiol. Rev. 14: 351–368

Macaskie LE, Empson RM, Lin F & Tolleys MR (1995) Enzymatically-mediated uranium accumulation and uranium re-

covery using a Citrobacter sp immobilised as a biofilm within a plug-flow reactor. J. Chem. Technol. Biotechnol. 63: 1–16

Macaskie LE, Lloyd JR, Thomas RAP & Tolley MR (1996) The use of microorganisms for the remediation of solutions contaminated with actinide elements, other radionuclides, and organic contaminants generated by nuclear fuel cycle activities. Nucl. Ener.-J., Brit. Nucl. Ener. Soc. 35: 257–271

Macaskie LE, Yong P, Doyle TC, Roig MG, Diaz M & Manzano T (1997) Bioremediation of uranium-bearing wastewater: biochemical and chemical factors influencing bioprocess application. Biotechnol. Bioengin. 53: 100–109

Madigan MT, Martinko JM & Parker J (2000). Brock Biology of Microorganisms, 9th edn. Prentice Hall, Upper Saddle River, NJ

Manning DAC & Bewsher A (1997) Determination of anions in landfill leachates by ion chromatography. J. Chromatogr. A 770: 203–210

McCarty PL (1972). Energetics of organic matter degradation. In Mitchell R. (Ed0.) Water Pollution Microbiology (pp 98–118). John Wiley and Sons, New York

Mikhailov VA (1973). Analytical Chemistry of Neptunium. Halsted Press, New York

Moskvin AI (1969) Complex formation of the actinides with anions of acids in aqueous solutions. Radiokhimiya 11: 458–460

Moulin VM, Moulin CM & Dran J-C (1996). Role of huminc substances and colloids in the behavior of radiotoxic elements in relation to nuclear waste disposal. In: Gaffney JS, Marley NA & Clark SB (Eds). Humic and Fulvic Acids: Isolation, Structure, and Environmental Role. American Chemical Society, Washington, DC, 651: 259–271

Nash KL, Morss LR, Jensen MP & Schmidt M (1996) Phosphate mineralization of actinides by measured addition of precipitating anions. Report CH2-6-C3-22, Chemistry Division, Argonne National Laboratory, Argonne, IL

National Research Council (2000a) Natural Attenuation for Groundwater Remediation, National Academy Press, Washington, DC

National Research Council (NRC) (2000b) Research Needs in Subsurface Science, US Department of Energy's Environmental Science Program, National Academy Press, Washington, DC

Nitsche H (1991) Basic research for assessment of geologic nuclear waste repositories: what solubility and speciation studies of transuranium elements can tell us. Materials Research Society Symposium Proceedings, Washington, DC

Nitsche H & Silva RJ (1996) Investigation of the carbonate complexation of Pu(IV) in aqueous solution. Radiochimica Acta 72: 65–72

Peck Jr HD (1993) Bioenergetic strategies of the sulfate-reducing bacteria. In: Odom JM & Singleton Jr R (Eds). The Sulfate-Reducing Bacteria: Contemporary Perspectives (pp 41–76). Springer-Verlag, New York

Phillips EJP, Landa ER & Lovley DR (1995) Remediation of uranium contaminated soils with bicarbonate extraction and microbial U(VI) reduction. J. Indust. Microbiol. 14: 203–207

Pokrovskii OS (2001) Speciation and activity coefficients of Am(III) nad Np(V) in seawater, Geochem. Inter. 39: 296–299

Pratopo RM, Moriyama H & Higashi K (1989) The behaviour of Neptunium under reducing conditions. Proceedings of the 1989 Joint International Waste Management Conference

Pratopo MI, Moriyama H & Higashi K (1990) Carbonate complexation of neptunium(IV) and analogous complexation of ground-water uranium. Radiochimica Acta 51: 27–31

Rai D, Swanson JL & Ryan JL (1987) Solubility of $NpO_2 \cdot xH_2O$ in the presence of Cu(I)/Cu(II) redox buffer. Radiochimica Acta 42: 35

Rai D, Rao L & Moore DA (1998) The influence of isosaccharinic acid on the solubility of Np(IV) hydrous oxide. Radiochimica Acta 83: 9–13

Read D, Ross D & Sims RJ (1998) The migration of uranium through Clashach sandstone: The role of low molecular weight organics in enhancing radionuclide transport. J. Contam. Hydrol. 38: 235–248

Reed DT, Zachara JM, Wildung RE & Wobber FJ (1991) Migration of radionuclides in geologic media: fundamental research seeds. Materials Research Society Symposium Proceedings, Washington, DC

Reed DT, Aase SB, Wygmans D & Banaszak JE (1998). The reduction of Np(V) and Pu(VI) by organic chelating agents. Radiochimica Acta 82: 109–114l

Reed DT, Vojta Y, Quinn JW & Richmann MK (1999) Radiotoxicity of plutonium(IV)-nitrilotriacitic acid complexes toward Chelatobacter heintzii. Biodegradation 10: 251–260

Riley RG, Zachara JM & Wobber FJ (1992) Chemical Contaminants on DOE lands and Selection of Contaminant Mixtures for Subsurface Science Research. DOE/ER-0547T, Office of Energy Research, US Department of Energy, Washington, DC

Rittmann BE & VanBriesen JM (1996) Microbiological processes in reactive transport modeling. In: Lichtner PC, Steefel CI & Oelkers EH (Eds). Reactive Transport in Porous Media. Mineralog. Soc. Amer. 34: 311–334

Rittmann BE & McCarty PL (2001) Environmental Biotechnology: Principles and Applications. McGraw-Hill, New York

Rittmann BE, Banaszak JE, VanBriesen JM, & Reed DT (2002) Mathematical modeling of precipitation and dissolution reactions in microbiological systems. Biodegradation 13: 239–250

Stockman HW (1998) Long-term modeling of plutonium solubility at a desert disposal site, including CO_2 diffusion, cellulose decay, and chelation. J. Soil Contamin. 7: 615–647

Stumm W & Morgan JJ (1996) Aquatic Chemistry, 3rd edn. John Wiley & Sons, New York

Thomas RAP & Macaskie LE (1996) Biodegradation of tributyl phosphate by naturally occurring microbial isolates and coupling to the removal of rranium from aqueous solution. Environ. Sci. Technol. 30: 2371–2375

VanBriesen JM & BE Rittmann (1999) Modeling speciation effects on biodegradation in mixed metal/chelate systems. Biodegradation 10: 315–330

VanBriesen JM & Rittmann BE (2000) Mathematical description of microbiological reactions involving intermediates. Biotechnol. Bioengineer. 67: 35–52

Weigel F, Katz JJ & Seaborg GT (1986) Plutonium. In: Katz JJ, Seaborg GT & Morss LR (Eds). The Chemistry of the Actinide Elements, 1 (pp 499–886). Chapman and Hall, New York

Yamaguchi T, Sakamoto Y & Ohnuki T (1994) Effect of the complexation on solubility of Pu(IV) in aqueous carbonate system. Radiochimica Acta 66/67: 9–14

Yong P & Macaskie LE (1995) Removal of the tetravalent actinide thorium from solution by a biocatalytic system. J. Chem. Technol. Biotechnol. 64: 87–95