

Characterization of Microbial Activities and U Reduction in a Shallow Aquifer Contaminated by Uranium Mill Tailings

D.A. Elias,¹ L.R. Krumholz,¹ D. Wong,¹ P.E. Long,² J.M. Suflita¹

¹ Institute for Energy and the Environment and the Department of Botany and Microbiology,
University of Oklahoma, Norman, OK 73019, USA

² Pacific Northwest National Laboratory, Richland, WA 99352, USA

Received: 26 August 2002; Accepted: 4 February 2003; Online publication: 21 May 2003

ABSTRACT

A characterization of the Shiprock, NM, uranium mill tailing site focused on the geochemical and microbiological factors governing *in-situ* uranium-redox reactions. Groundwater and aqueous extracts of sediment samples contained a wide concentration range of sulfate, nitrate, and U(VI) with median values of 21.2 mM, 16.1 μ M, and 2.7 μ M, respectively. Iron(III) was not detected in groundwater, but a median value of 0.3 mM in sediment extracts was measured. Bacterial diversity down gradient from the disposal pile reflected the predominant geochemistry with relatively high numbers of sulfate- and nitrate-reducing microorganisms, and smaller numbers of acetogenic, methanogenic, nitrate-dependent Fe(II)-oxidizing, Fe(III)-reducing, and sulfide-oxidizing bacteria. In aquifer slurry incubations, nitrate reduction was always preferred and had a negative impact on sulfate-, Fe(III)-, and U-reduction rates. We also found that sulfate-reduction rates decreased sharply in the presence of clay, while Fe(III)-reduction increased with no clear impact on U reduction. In the absence of clay, iron and sulfate reduction correlated with concentrations of Fe(III) and sulfate, respectively. Rates of U(VI) loss did not correlate with the concentration of any electron acceptor. With the exception of Fe(III), electron donor amendment was largely unsuccessful in stimulating electron acceptor loss over a 2-week incubation period, suggesting that endogenous forms of organic matter were sufficient to support microbial activity. Our findings suggest that efforts to accelerate biological U reduction should initially focus on stimulating nitrate removal.

Introduction

Contamination of the subsurface with uranium, heavy metals, and radionuclides is of concern both to the environment and to human health. When soluble uranium

reaches the subsurface, it migrates with groundwater to potable water bodies and can biomagnify in food chains [20, 39]. The reduced form of uranium (U(IV)) is insoluble and of far less environmental concern [23, 40]. Microorganisms capable of U reduction include fermenters such as *Clostridium* sp. [13], Fe(III) reducers such as *Shewanella putrefaciens* [14, 26] and *Geobacter metallireducens* [27],

the sulfate-reducing bacteria *Desulfovibrio* (*Dsv.*) *desulfuricans* [14, 28] and *Desulfotomaculum reducens* [37], and a denitrifying *Pseudomonas* sp. [4]. Therefore, microbial U reduction is often considered an important remedial option in contaminated locales.

However, the potential for uranium bioremediation is a function of multiple interacting factors. These include the presence of suitable microorganisms, and the availability of electron acceptors and donors, as well as sediment components that may complex with uranium [29]. Subsurface microbial communities often exhibit a preference in electron-accepting processes when several options are available. For instance, nitrate reduction tends to predominate when available [32], followed by Fe(III) and then sulfate reduction [9]. It is unknown how U reduction might integrate into such generalizing patterns of microbial activity.

The addition of electron donors has been used to differentially stimulate particular microbial respiratory processes. For instance, the use of methanol to stimulate methanogenesis even in the presence of high levels of sulfate has been known for many years [21]. The electron donors that might favor U reduction are largely unknown, although a recent report showed stimulation of both Fe(III) and U(VI) reduction, but not sulfate reduction, through the addition of 100 μ M acetate [17]. The relevance of such small amendments relative to the large endogenous pool of available electron donors in contaminated areas is at least debatable. However, such treatments can sometimes serve to implicate the kinds of microorganisms that can be stimulated to catalyze U reduction.

In addition to the aforementioned factors, subsurface strata can affect microbial activity in various ways. Clay layers harbor lower numbers of culturable bacteria than sandy sediments and generally exhibit lower electron acceptor transformation rates [8, 37]. Clay can also limit the bioavailability of U(VI) [28] via sequestration [12].

Although the above factors may be evaluated individually, study of their complex interplay at contaminated sites is far more realistic. A Uranium Mill Tailings Remedial Action (UMTRA) site in Shiprock, NM, was chosen for this investigation. This site is known to be U-contaminated and also serves as a model for other contaminated Department of Energy lands. The site is a former uranium mill of 230 acres (1954–1973) where wastes associated with U mining and processing were consolidated into a mill tailings disposal pile covered with 2.2 m of compacted clay as a radon barrier and a rock layer to prevent erosion (<http://www.em.doe.gov/>

bemr96/ship.html: <http://www.doeal.gov/oepm/factshp.htm>). Surface cleanup of the site was completed in 1996, and the site is currently under the care of the Department of Energy (DOE).

We characterized the subsurface microbial community and the potential for *in situ* U reduction relative to other processes. We also attempted to evaluate the impact of lithology and exogenous electron donor amendments on U reduction. The study of a real contaminated site helps provide practical insights on the steps needed for U bioremediation.

Materials and Methods

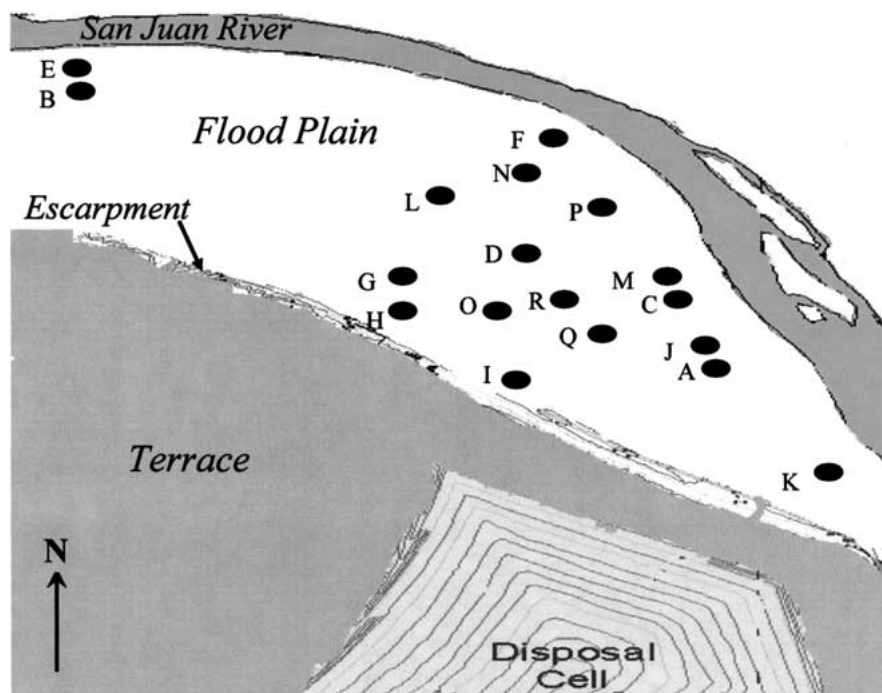
Sample Collection

Alluvial floodplain sediments adjacent to the UMTRA disposal cell in Shiprock, NM, were collected between October 1998 and April 2000 (Fig. 1). Samples were obtained from different depths in largely sand or sand–clay sediments, as well as in a Mancos shale formation, using augers, backhoes, and rotasonic drilling equipment [7, 17, 18]. A backhoe was used to collect bulk sediment samples, while the drilling equipment afforded intact core recovery from discrete depths. A total of 36 sediment samples were obtained and analyzed (Fig. 1). Groundwater was collected as previously described [7] from 28 existing wells using a peristaltic pump and placed in sealed sterile 1-L bottles. All samples were placed under argon and refrigerated at 4°C until they were used.

Geochemical Characteristics

Initial characterization of the Shiprock site began with a measurement of the geochemical constituents important for supporting microbial activity. Analysis of groundwater involved centrifugation (27,000 g, 20 min, 10°C) to remove solid particles, whereas aqueous extracts of sediment samples were obtained by mixing sediment (1 g) with anaerobic water (10 mL) and centrifuging (27,000 g, 20 min, 10°C). Chloride, nitrate, and sulfate concentrations for all samples were determined using a Dionex DX 500 ion chromatograph with an AS4A anion column. Levels of Fe(II)/Fe(III) were determined by the colorimetric method described previously [24].

Samples were prepared for U(VI) analysis by centrifugation of sediments (27,000 g, 20 min, 10°C) and filtering (0.45 μ m) the porewater supernatant. This was followed by acidification with 10% (v:v) HNO_3 and boiling to dryness. The residue was then resuspended in 1 M HNO_3 to ensure that there was no sequestration or photoreduction of the uranium [5]. The levels of U(VI) were measured using a kinetic phosphorescence analyzer (Model KPA-11, Chemchek Instruments, Inc.) as described previously [5, 29].



Sample Location	Depth (m)	Lithology
A	4.0, 4.6, 4.7	Sand/clayey
B	2.6, 4.0	Sand
C	4.0, 5.4	Sand/clayey
D	2.9	Sand
E	2.9	Sand
F	2.9	Sand
G	2.9	Sand
H	2.9	Sand
I	2.9	Sand
J	2.9	Sand
K	2.9	Sand
L	2.9	Sand
M	2.9	Sand
N	2.6, 4.1, 4.6	Sand
O	2.0, 3.4, 4.6, 4.9, 5.1	Sand/clayey
P	1.6, 3.4, 4.4, 4.6, 5.3	Sand/clayey
Q	3.0, 3.6, 4.4	Sand/clayey
R	7.7, 12.9, 17.9	Shale

Fig. 1. A schematic map of the Shiprock, NM, UMTRA disposal site showing sediment collection locations in the alluvial floodplain (letters). The accompanying table indicates the depth and predominant lithology of the samples. Samples containing an obvious clay component are noted in bold text.

Bacterial Enumerations

In order to assess the types of bacteria within the subsurface community, a variety of microorganisms were enumerated from sediment samples using a three-tube most probable number (MPN) assay [36]. Sediment from locales directly down gradient of the disposal pile were used to determine which bacterial groups were dominant in this highly contaminated area. All tubes were incubated at room temperature (approximately 20°C) in the dark for 6 weeks before being scored for both growth and loss of electron acceptor. Sulfate- [36] and nitrate [3]-reducing bacteria were enumerated using media previously described. The loss of sulfate and nitrate was determined after 6 weeks by centrifugation (27,000 g, 20 min, 10°C) of a culture medium aliquot followed by ion chromatography as above. Acetogenic bacteria were

quantified using media previously described [33] with acetate production determined by gas chromatography [19]. Fe(III)-reducing bacteria were cultured using the described medium [25] with the loss of Fe(III) and consequent increase in Fe(II) determined using the colorimetric assay [24] designed for that purpose. Nitrate-dependent, Fe(II)-oxidizing bacteria were grown [6] and activity verified by determining the loss of Fe(II) and increase in Fe(III), as well as the loss of nitrate as above. Aerobic Fe(II)-oxidizing bacteria were estimated using a previously described medium [33] with the loss of Fe(II) and increase in Fe(III) determined as above. Sulfide oxidizing bacteria were enumerated [35] and analyzed for sulfide loss by centrifuging an aliquot of medium followed by quantitation using the colorimetric assay [10]. The methanogen population was determined using the coenzyme M assay [11].

Effect of Nitrate on Electron Accepting Activity

When several potential electron acceptors are available in the subsurface, nitrate is thermodynamically preferred [32, 34]. Hence, we tested if this was the case in Shiprock sediments, and what effect the presence of nitrate might have on sulfate, Fe(III), and U(VI) reduction rates. Slurry incubations contained sediment (20 g) and filter-sterilized groundwater (50 mL) under a N₂/CO₂ atmosphere. These included an autoclaved negative control and a nonsterile sample amended with uranyl carbonate (2 mM) to ensure that sufficient U(VI) was available. Uranyl carbonate preparation was modified from a previous report [27] by boiling 2 mM uranyl acetate/30 mM NaHCO₃ solution to dryness and resuspending the precipitate in the same volume of 30 mM NaHCO₃. The resulting acetate concentration in the solution (370 μM) was measured as described above. Electron acceptor concentrations were periodically determined over a 156-d incubation by aseptically removing groundwater (1 mL), centrifuging (22,700 g, 20 min, 10°C) to remove solid material, and analyzing as above. In addition, the production of sulfide was quantified using a passive extraction procedure [37] and colorimetric assay [10]. All incubations were at room temperature in the dark and in triplicate. Rates of reduction were determined by using data points in the apparent zero-order portion of the progress curve that yielded a straight line, and then normalized for both the weight of sediment used and the length of incubation.

Effect of Clay on Electron-Accepting Activity

Sediment cores from various depths were obtained in the drilling operation and used to test the influence of clays on sulfate, Fe(III), and U(VI) reduction rates. Samples containing large concentrations of clays are known to inhibit sulfate reduction [37]. Subsamples from the cores were obtained using sterile knives where changes in the sediment lithology were visually obvious. Incubations were as above except that no exogenous uranium was added. The sediments were sacrificed periodically for electron acceptor measurements as described above, with the final samples being analyzed after 24 d.

Effect of Electron Donor Amendments

Slurry incubations were constructed from sediment samples containing low (5 μM) and high (0.3 mM) nitrate levels, and amended with various electron donors. This was done in an effort to stimulate *in situ* uranium reduction and discern if sufficient endogenous electron donor was available to support microbial activity. We equated U(IV) precipitation with the reduction of U(VI) to U(IV) [14]. Electron acceptor concentrations were periodically determined as described above. Electron donor concentrations were monitored using an AS-11A column on the Dionex 500 ion chromatograph. The incubations consisted of an autoclaved negative control, sediment amended with 2 mM uranyl carbonate to quantitate endogenous levels of uranium reduction, and amendments of 2 mM uranyl carbonate +20 mM

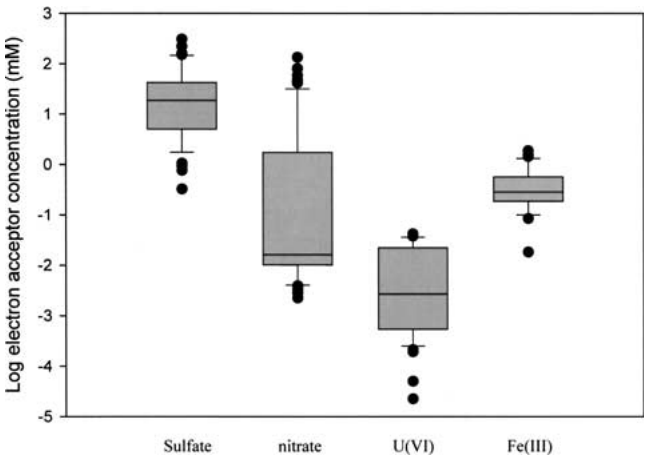


Fig. 2. Box and whisker plots for sulfate, nitrate, U(VI) in groundwater (28 samples), and aqueous sediment extracts (36 samples). Levels of Fe(III) are for the latter samples only. The top and bottom of each box represent the 75th and 25th percentiles, respectively. The middle line of the box represents the median (59th percentile). The whiskers at the top and bottom of the box represent the 10th and 90th percentiles, respectively. The circles represent the 10% lowest and highest electron acceptor concentrations observed.

sodium acetate, sodium formate, sodium lactate, ethanol, or methanol. Samples were incubated for 32 d.

Results

Geochemical Characteristics

Electron acceptor concentrations measured in groundwater and aqueous sediment extracts spanned extremely large ranges (Fig. 2). Sulfate was quantitatively the dominant electron acceptor with a median concentration of 21.2 mM, while nitrate, Fe(III), and U(VI) concentrations had median values of 16.1 μM, 0.3 mM, and 2.7 μM, respectively (Fig. 2). Nitrate exhibited a wider concentration range relative to other potential electron acceptors. That is, samples ranged from below detection to greater than 134 mM. Not surprisingly given the relatively poor aqueous solubility of iron at neutral pH values [21], Fe(III) was below detection in all groundwater samples and could only be measured in the aqueous sediment extracts up to a maximum of 1.9 mM. The U(VI) concentration ranged from below detection to 42 μM.

Bacterial Enumerations

This basic geochemical profile was largely consistent with microbial enumeration assays. Interrogation of several

samples with depth along the groundwater flowpath revealed that sulfate-reducing bacteria vary from 2.0×10^1 to 4.3×10^4 cells g^{-1} sediment with comparable numbers for nitrate reducers (9.3×10^1 to 2.3×10^4 cells g^{-1}) (data not shown). Other bacterial types were also found including acetogens (10^3 cells g^{-1}), methanogens (10^3 cells g^{-1}), and both aerobic (4 cells g^{-1}) and anaerobic (10 cells g^{-1}) Fe(II)-oxidizing organisms. The Fe(III)-reducing bacteria in both the groundwater and sediments were either below the detection limit or at very low values (4 cells g^{-1}). This degree of microbial diversity is not unusual in sedimentary systems and is consistent with previous findings on the importance of sulfate-reducing bacteria [7] at the Shiprock UMTRA site. However, the high degree of variability associated with electron acceptor availability does not preclude the possibility of localized areas where the dominant microbial populations can vary substantially. This point notwithstanding, it is well recognized that the presence and abundance of microorganisms does not necessarily correlate with their *in situ* activity. Consequently, our efforts were focused on the determination of the predominant electron-accepting activity in samples obtained from the site.

Effect of Nitrate on Electron-Accepting Activity

Even though nitrate was not the dominant electron acceptor, in all cases it was preferentially consumed over sulfate, Fe(III), and U(VI). The latter electron acceptors were not rapidly consumed until nitrate was nearly depleted (usually in ~ 2 d; data not shown). The impact of nitrate can be seen by a comparison of the sulfate, Fe(III), and U(VI) reduction rates before and after nitrate consumption in sediment samples (Fig. 3). The sulfate loss rate ranged from 0 to $2.9 \mu\text{mol g}^{-1} \text{d}^{-1}$ with a median value of $0.2 \mu\text{mol g}^{-1} \text{d}^{-1}$ when nitrate was greater than $5 \mu\text{M}$ in the incubations (Fig. 3). When nitrate fell below detection limits ($5 \mu\text{M}$), sulfate-reduction rates increased to a median of $2.7 \mu\text{mol g}^{-1} \text{d}^{-1}$. A similar but less drastic effect was seen for the Fe(III) and U reduction rates. These rates increased in the absence of nitrate from a median value of 1.3 to $4.2 \text{ nmol g}^{-1} \text{d}^{-1}$ for Fe(III), while comparable measures for U(VI) increased from 0.11 to $0.21 \text{ nmol g}^{-1} \text{d}^{-1}$.

Effect of Clay on Electron-Accepting Activity

Of the 36 sediment samples taken from the Shiprock UMTRA site, 24 had a substantial amount of nitrate, while

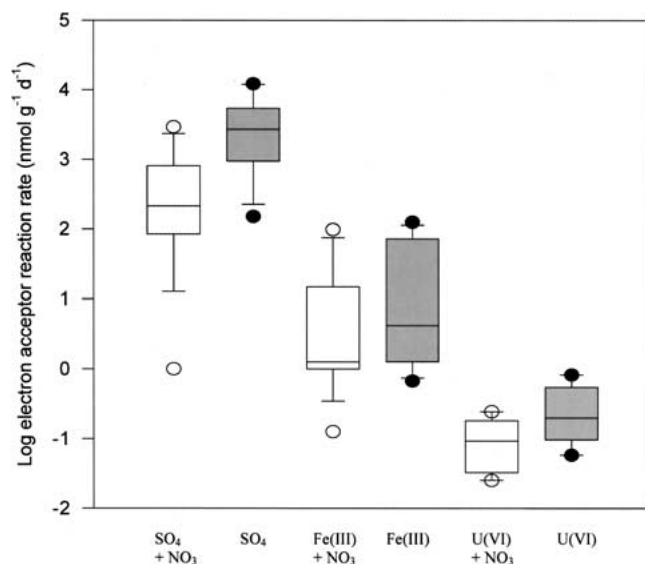


Fig. 3. Box and whisker plots comparing the rate of sulfate, Fe(III), and U(VI) reduction before (+NO₃) and after (shaded) the microbial consumption of endogenous levels of nitrate in 36 sediment slurry incubations.

16 had little or no endogenous level of this anion. Hence, without the overwhelming influence of nitrate, this subset of 16 samples that were collected together was used to consider other environmental factors influencing electron acceptor reduction. Once again, rates of microbial activity varied considerably for sulfate (0 – $112 \mu\text{mol g}^{-1} \text{d}^{-1}$), Fe(III) (21 – $226 \text{ nmol g}^{-1} \text{d}^{-1}$), and U(VI) (0.02 – $0.63 \text{ nmol g}^{-1} \text{d}^{-1}$) with median values of $7.7 \mu\text{mol g}^{-1} \text{d}^{-1}$ and 46.4 and $0.22 \text{ nmol g}^{-1} \text{d}^{-1}$, respectively.

These rates were compared with the concentration of the respective electron acceptor in the sample (Fig. 4). We found a correlation between the sulfate concentration and the rate of sulfate reduction (Fig. 4A) ($r^2 = 0.831$). However, this correlation was based only on samples that exhibited a measurable rate of sulfate reduction. Seven of the 16 samples exhibited insignificant levels of sulfate loss, and each was found to be predominantly clay. This was reflected in the median reaction rate decreasing from $21.4 \mu\text{mol g}^{-1} \text{d}^{-1}$ in sandy sediments to $7.7 \mu\text{mol g}^{-1} \text{d}^{-1}$ when clay containing sediments were included in the dataset.

A similar trend was noted for Fe(III) reduction ($r^2 = 0.839$) (Fig. 4B). However, these rates generally increased in clay-containing samples (median, $46.4 \text{ nmol g}^{-1} \text{d}^{-1}$) relative to sandy sediments (median, $28.5 \text{ nmol g}^{-1} \text{d}^{-1}$). In contrast to both sulfate and Fe(III) reduction, the relationship between U reduction and U(VI) concentrations in either the presence or absence of clay was not

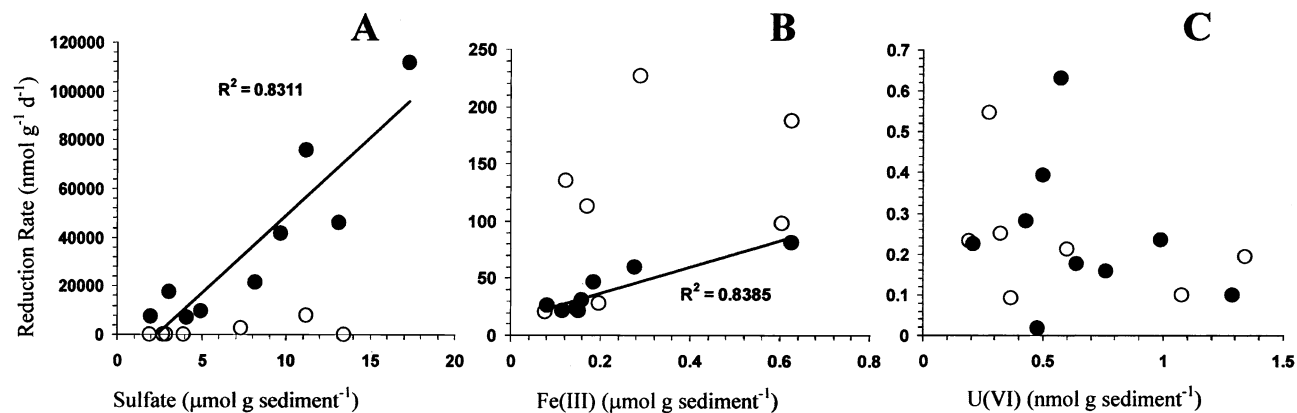


Fig. 4. The relationship between electron acceptor concentration and the corresponding reaction rates for sulfate (A), Fe(III) (B), and uranium(VI) (C) in samples with little or no nitrate. Samples where clay was present (○) can be distinguished from those where clay was not evident (●).

obvious (Fig. 4C). The range and median value of U(VI) loss was similar in clay samples (0.09–0.55 nmol g⁻¹ d⁻¹, median 0.21 nmol g⁻¹ d⁻¹) to those in sandy sediments (0.02–0.63 nmol g⁻¹ d⁻¹, median 0.23 nmol g⁻¹ d⁻¹).

Effect of Electron Donor Amendments

The relationship between electron-accepting processes and their respective electron acceptor concentrations suggests that the latter exerts an important influence on the former, and that the availability of electron donors is not a limiting factor. To test this hypothesis, we amended sediment slurries with 20 mM acetate, ethanol, formate, lactate, and methanol to incubations that contained high (0.3 mM) and low (5 μM) nitrate concentrations. High-performance liquid chromatography (HPLC) analysis revealed that these donors were largely consumed within 16 d. After this period, 3 mM methanol and 2 mM ethanol could be detected in the low nitrate samples while the other electron donors were consumed to below detection. Once again, nitrate was removed preferentially when it was present (data not shown). There was also a substantial amount of electron acceptor consumption in the donor-unamended controls (Table 1). During the course of this experiment, sulfate was reduced from 9.9 mM to 3.9 mM in the low-nitrate incubations compared to a decrease from 21.3 mM to 8.3 mM in the high-nitrate samples. This represents a 39% reduction in each case. A similar loss of Fe(III) was measured in the donor-unamended controls in both low (from 0.68 to 0.42 mM) and high (from 0.64 to 0.45 mM) nitrate sediments. The percentage of U(VI) reduced was greater in low-rather than high-nitrate samples even

though both had starting concentrations of 160 μM U(VI), consistent with the inhibitory influence of this nitrate.

In most cases, the exogenous addition of an electron donor did not significantly stimulate sulfate reduction over the endogenous controls (Table 1). Notable exceptions to this include lactate and ethanol. The amendments did stimulate Fe(III) reduction to a statistically significant extent regardless of the initial nitrate concentration in the incubations. However, since iron is a far less quantitatively important electron acceptor, this stimulation could only account for 0.16 to 1.0 mM electron donor consumption. The same electron donor treatments had relatively little influence on U reduction with the possible exception of methanol. Collectively these findings suggest that the endogenous amounts of electron donors are not limiting in these incubations. Further, the consumption of exogenous forms of organic matter will likely be coupled with the preferential metabolism of nitrate before other electron acceptors are utilized to a substantial degree.

Discussion

Investigation of the geochemical parameters at the Shiprock site showed a substantial variability in several potential electron acceptors including sulfate, nitrate, Fe(III), and U(VI). A comparison of these levels showed that sulfate was by far quantitatively dominant, followed by the three latter electron acceptors. While median values for all but nitrate are above safe drinking water levels as outlined by the US EPA (<http://www.epa.gov/safewater/mcl.html>), several locations on the alluvial floodplain contained

Table 1. Percent of electron acceptor remaining in sediment slurries after 16 d incubation

Electron-donor amendment	High-nitrate slurry			Low-nitrate slurry		
	Sulfate	Fe(III)	U(VI)	Sulfate	Fe(III)	U(VI)
None	61	71	57	61	62	16
Acetate	57	35 ^a	62	50	19 ^a	20
Ethanol	50 ^a	6 ^a	56	7 ^a	38 ^a	22
Formate	58	0 ^a	64	57	39 ^a	18
Lactate	47 ^a	0 ^a	63	41 ^a	0 ^a	21
Methanol	57	0 ^a	74	78	0 ^a	4 ^a

^a A positive statistical difference from unamended control using ANOVA analysis ($p < 0.05$)

greater than 800 times the standard for the latter anion. Although these levels make this area unsuitable as a source of potable water, there is an abundance of electron acceptor available for microbial respiratory processes.

Bacterial enumerations indicated high numbers of nitrate- and sulfate-reducing bacteria that appear to dominate a diverse microbial community. These results are consistent with previous findings at the Shiprock site [14]. Further, since these and other types of microorganisms have been implicated in U reduction [1, 4, 26, 28, 30], *in situ* microbial U precipitation would appear possible.

Sediment slurries were used to evaluate the reduction rates associated with each of the electron acceptors. This methodology effectively reduces small-scale variability by homogenizing the samples, and *in situ* reduction rates may be locally different. However, the respective rates for each process revealed that nitrate loss was preferred whenever this anion was available. Subsequently, the loss of sulfate, Fe(III), and U reduction occurred concomitantly, clearly demonstrating the negative impact of nitrate on the latter processes. This effect was verified by substantial increases in electron acceptor reduction activity after nitrate was exhausted. The favored reduction of nitrate has been shown previously [32, 34] and agrees with thermodynamic considerations, especially in sulfate-reducing bacteria where U(VI) is favored over sulfate [22]. Since ammonium, but no nitric acid was used in the uranium milling process [2], ammonium oxidation would seem to be the likely source of high levels of nitrate contamination at the site [18]. We found evidence for both ammonium-oxidizing and nitrate-reducing populations corroborating earlier work [18], suggesting an active cycling of nitrogenous compounds. One consequence of this cycling is that the intermediates of nitrate reduction could oxidize U(IV) [31], making nitrogenous compound removal critical to ultimate remediation of U contamination at the site.

In the absence of nitrate, sulfate and Fe(III) reduction rates were correlated with the concentration of their respective electron acceptors (Fig. 4). No such relationship could be seen for U. The presence of clay proved to be an interdicting factor in both of the former relationships by inhibiting sulfate-reducing bacteria. The inhibition of Fe(III) and sulfate reduction by clay has been seen previously [38]. Cessation of sulfate reduction in clay-containing samples may have allowed for the observed increase in Fe(III)-reduction rates due to a lack of competition for available electron donors.

The availability of endogenous electron donors was likely sufficient to support microbial activity since the addition of exogenous electron donors did not generally stimulate overall respiratory processes. Only lactate and ethanol stimulated sulfate reduction, while the increase in Fe(III) loss with all five electron donors would at best only account for a minor fraction of the available electron donor. Although we attempted to discern whether the amendments also increased nitrate reduction, the high endogenous rate of this process precluded this determination. The lack of U reduction stimulation with excess exogenous, as well as endogenous, electron donors suggests that longer incubation periods will likely be necessary before such an amendment approach is feasible. These results contrasts with a recent report where Fe(III) and U(VI) reduction were differentially stimulated relative to the loss of sulfate [37]. However, the latter experiments were performed on samples from a single locale on the floodplain. This is probably an example of an area that differs from the more generalizing site characteristics noted here and such areas are expected when such large variability in geochemical and microbiological properties are encountered.

Thus it would seem essential to recognize that nitrate- and sulfate-reducing bacteria are important microorganisms at this site. The dissimilatory sulfite reductase (DSR) gene of *Desulfotomaculum* was found at many areas of the

site with a positive correlation between signal strength and the U(VI) concentration [7], where U(VI) could be acting as electron acceptor to stimulate the growth of these organisms. In addition, several biomarkers consistent with *Desulfovibrio*-like signals were found in areas of the floodplain where we have previously observed active microbial U reduction, which could account for the relatively low U(VI) concentrations in those specific locations (Elias D, Wong D, Senko J, Long PE, McKinley JP, Suflita JM, Krumholz LR (2000) EOS, Trans Am Geophys Union Fall Meet 81: F214).

It would also appear that efforts to stimulate *in situ* U bioremediation should first focus on nitrate removal by either biotic or abiotic mechanisms. As a more favored thermodynamic process, nitrate reduction should predominate until it is removed from the contamination area. Although there is adequate endogenous electron donor to drive relatively short-term incubations, a longer term approach with electron donor addition for *in situ* treatment would clearly be appropriate.

Acknowledgments

We thank John Senko for analysis of uranium samples. Support of the US Department of Energy, Office of Science, Biological and Environmental Research under the Natural and Accelerated Bioremediation Research (NABIR) Program is gratefully acknowledged. The cooperation of the US Department of Energy Uranium Mill Tailings Remedial Action (UMTRA) Program was crucial to the success of this research.

References

1. Abdelouas A, Lu Y, Lutze W, Nuttall HE (1998) Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water. J Contam Hydrol 35:217–233

2. Anonymous (1996) Final programmatic environmental impact statement for the uranium mill tailings remedial action ground water project. US Department of Energy DOE/EIS-0198. <http://www.doegjpo.com/gwwp/shp/shiprock.htm>.

3. Baldensperger JB (1978) An iron-containing superoxide dismutase from the chemolithotrophic *Thiobacillus denitrificans* “RT” strain. Arch Microbiol 119:237–244

4. Barton LL, Choudhury K, Thomson BM, Steenhoudt K, Groffman AR (1996) Bacterial reduction of soluble uranium: The first step of *in situ* immobilization of uranium. Radioact Waste Manag Environ Restorat 20:141–151

5. Brina R, Miller AG (1992) Direct detection of trace levels of uranium by laser-induced kinetic phosphorimetry. Anal Chem 64:1413–1418

6. Caldwell ME, Tanner RS, Suflita JM (1999) Microbial metabolism of benzene and the oxidation of ferrous iron under anaerobic conditions: Implications for bioremediation. Anaerobe 5:595–603

7. Chang Y, Peacock AD, Long PE, Stephen JR, McKinley JP, MacNaughton SJ, Anwar-Hussain AKM, Saxton AM, White DC (2001) Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. Appl Environ Microbiol 67:3149–3160

8. Chapelle FH, Lovley DR (1990) Rates of microbial metabolism in deep coastal plain aquifers. Appl Environ Microbiol 56:1865–1874

9. Chapelle FH, Lovley DR (1992) Competitive exclusion of sulfate reduction by iron(III)-reducing bacteria: a mechanism for producing discrete zones of high-iron ground water. Ground Water 30:29–36

10. Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

11. Elias DA, Krumholz LR, Tanner RS, Suflita JM (1999) Estimation of methanogen biomass by quantitation of coenzyme M. Appl Environ Microbiol 65:5541–5545

12. Elias DA, Senko JM, Krumholz LR (2003) A procedure for quantitation of total oxidized uranium for bioremediation studies. J. Microbiol. Meth. 53:343–353

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

14. Ganesh R, Robinson KG, Reed GD, Sayler GS (1997) Reduction of hexavalent uranium from organic complexes by sulfate- and iron-reducing bacteria. Appl Environ Microbiol 63:4385–4391

15. Gorby YA, Lovley DR (1992) Enzymatic uranium precipitation. Environ Sci Technol 26:205–207

16. Gruntzig V, Nold SC, Zhou J, Tiedje JM (2001) *Pseudomonas stutzeri* nitrite reductase gene abundance in environmental samples measured by real-time PCR. Appl Environ Microbiol 67:760–768

17. Holmes DE, Finneran KT, O’Neil RA, Lovley DR (2002) Enrichment of members of the family Geobacteraceae associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. Appl Environ Microbiol 68:2300–2306

18. Ivanova IA, Stephen JR, Chang Y, Brüggemann J, Long PE, McKinley JP, Kowalchuk GA, White DC, Macnaughton SJ (2000) A survey of 16S rRNA and amoA genes related to autotrophic ammonia-oxidizing bacteria of the beta-subdivision of the class proteobacteria in contaminated groundwater. Can J Microbiol 46:1012–1020

19. Jackson BE, McInerney MJ (2002) Anaerobic microbial metabolism can proceed close to thermodynamic limits. Nature 415:454–456

20. Janssen MPM (1993) Biomagnification of metals in terrestrial ecosystems. Sci Total Environ Supp:1–511

21. King GM (1984) Utilization of hydrogen, acetate, and “non-competitive” substrates by methanogenic bacteria in marine sediments. *Geomicrobiol J* 3:275–306
22. Langmuir D (1997) *Aqueous Environmental Geochemistry*. Prentice Hall, Saddle River, NJ, pp 496–500
23. Lovley DR, Coates JD (1997) Bioremediation of metal contamination. *Curr Opin Biotechnol* 8:285–289
24. Lovley DR, Phillips EJP (1987) Rapid assay for microbially reducible ferric iron in aquatic sediments. *Appl Environ Microbiol* 53:1536–1540
25. Lovley DR, Phillips EJP (1988) Novel mode of microbially energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl Environ Microbiol* 54:1472–1480
26. Lovley DR, Phillips EJP (1992) Reduction of uranium by *Desulfovibrio desulfuricans*. *Appl Environ Microbiol* 58:850–856
27. Lovley DR, Phillips EJP, Gorby YA, Landa ER (1991) Microbial reduction of uranium. *Nature* 350:413–417
28. Lovley DR, Roden EE, Phillips EJP, Woodward JC (1993) Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Mar Geol* 113:41–53
29. McKinley JP, Zachara JM, Smith S, Turner GD (1995) The influence of uranyl hydrolysis and multiple site-binding reactions on adsorption of U(VI) to montmorillonite. *Clays Clay Miner* 43:586–598
30. Mohagheghi A, Updegraff DM, Goldhaber MB (1984) The role of sulfate-reducing bacteria in the deposition of sedimentary uranium ores. *Geomicrobiol J* 4:153–173
31. Senko JM, Istok JD, Suflita JM, Krumholz LR (2002) In-situ evidence for uranium immobilization and remobilization. *Environ Sci Technol* 36:1491–1496
32. Smith RL (2002) Determining the terminal electron-accepting reaction in the saturated subsurface. In: CJ Hurst (ed) *Manual of Environmental Microbiology*, 2nd ed. American Society for Microbiology, Washington, DC, pp 743–752
33. Sneath PH, Skerman VB (1966) A list of type and reference strains of bacteria. *Int J Syst Bacteriol* 16:1–133
34. Stumm W, Morgan JJ (1996) Oxidation and reduction; equilibria and microbial mediation. Chapter 8 in: *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, 3rd ed. John Wiley & Sons, New York, pp 425–513
35. Sugio T, White KJ, Shute E (1992) Existence of a hydrogen sulfide: ferric ion oxidoreductase in iron-oxidizing bacteria. *Appl Environ Microbiol* 58:431–433
36. Tanner RS (1996) Monitoring sulfide and sulfate-reducing bacteria. *Proceedings of the Fifth International Conference on Microbially Enhanced Oil Recovery and Related Biotechnology for Solving Environmental Problems*. NTIS, Springfield, VA
37. Tebo BM, Obraztsova AY (1998) Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiol Lett* 162:193–198
38. Ulrich GA, Martino D, Burger K, Routh J, Grossman EL, Ammerman JW, Suflita JM (1998) Sulfur cycling in the terrestrial subsurface: Commensal interactions, spatial scales, and microbial heterogeneity. *Microbial Ecol* 36:141–151
39. vanStraalen NM, Ernst W (1991) Metal biomagnification may endanger species in critical pathways. *Oikos* 62:255–257
40. White C, Sayer JA, Gadd GM (1997) Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiol Rev* 20:503–516