Available online at www.sciencedirect.com
ScienceDirect
Applied Geochemistry 21 (2006) 1539–1550
Applied Geochemistry
www.elsevier.com/locate/apgeochem

The biogeochemical behaviour of U(VI) in the simulated near-field of a low-level radioactive waste repository

James R. Fox [a], Robert J.G. Mortimer [a], Gavin Lear [b], Jonathan R. Lloyd [b], Ian Beadle [c], Katherine Morris [a,*]

[a] *Institute of Geological Sciences, School of Earth and Environment, University of Leeds, Leeds LS2 9JT, UK*
[b] *Williamson Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, the University of Manchester, Manchester M13 9PL, UK*
[c] *Nexia solutions, BNFL plc, Risley, Warrington WA3 6AS, UK*

Received 29 September 2005; accepted 18 May 2006
Editorial handling by R. Zielinski
Available online 14 August 2006

Abstract

Microbial processes have the potential to affect the mobility of radionuclides, including U in radioactive wastes. A range of geochemical, molecular biological and mineralogical techniques were applied to investigate stable element biogeochemistry and U solubility in the simulated "near-field" (or local environment) of a low-level radioactive waste (LLW) repository. The experiments used a microbial inoculum from the trench disposal area of the UK LLW repository at Drigg, Cumbria, England, in combination with a synthetic trench leachate representing the local environment at the Drigg site. In batch culture experiments in the absence of U, a classic redox progression of terminal electron accepting processes (TEAPs) occurred in the order NO_3^-, Fe(III) and SO_4^{2-} reduction. When 126 μM U was added to the system as $U(VI)_{aq}$, up to 80% was reduced to U(IV) by the indigenous microbial consortium. The U(IV) was retained in solution in these experiments, most likely by complexation with citrate present in the experimental medium. No $U(VI)_{aq}$ was reduced in sterile cultures, confirming that $U(VI)_{aq}$ reduction was microbially mediated. Interestingly, when $U(VI)_{aq}$ was present, the progression of TEAPs was altered. The rate of Fe(III) reduction slowed compared to experiments without $U(VI)_{aq}$, and SO_4 reduction occurred at the same time as U(VI) reduction. Finally, an experiment where SO_4^{2-}-reducing microorgansisms were inhibited by Na molybdate showed no ingrowth of sulfide minerals, but U(VI) reduction continued in this experiment. This suggested that sulfide minerals did not play a significant role in abiotically reducing U(VI) in these systems, and that metal-reducing microorganisms were dominant in mediating U(VI) reduction. Bacteria closely related to microorganisms found in engineered and U-contaminated environments dominated in the experiments. Denaturing gradient gel electrophoresis (DGGE) on 16SrRNA products amplified from broad specificity primers showed that after incubation, differences in diversity and abundance of the microbial culture were observed between U and non-U experiments. These results indicate that the biogeochemistry of the LLW repository near-field stimulates reduction of $U(VI)_{(aq)}$ to U(IV) under anaerobic conditions and that the fate of reduced U(IV) will depend on the complexants present in LLW systems.

* Corresponding author.
E-mail address: k.morris@see.leeds.ac.uk (K. Morris).

0883-2927/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apgeochem.2006.05.006

1. Introduction

The Drigg low-level waste repository, Cumbria, England, is the principal disposal site for low-level radioactive waste (LLW) in the UK (BNFL, 2002). This near surface disposal facility contains over 900,000 m^3 LLW. Originally waste was loose tipped into shallow "trenches", with more recent disposals being grouted and placed in engineered concrete vaults. The focus of this study is the biogeochemical environment within the "near-field" of the Drigg trenches. The Drigg trenches contain substantial quantities of cellulose, soil and metals. Overall, the site contains a heterogeneous mix of these materials, has an average annual temperature of 10 °C, and is microbially active with CH_4 and CO_2 detected at various locations on site, and with volatile fatty acids present in trench leachates (Humphreys et al., 1997). Thus, the Drigg trench environment is a low-level waste "landfill" containing electron donor rich waste and leachate and a heterogeneous mix of terminal electron acceptors including NO_3^-, Fe(III) and SO_4^{2-}. This high electron donor content is in contrast to many sub-surface aquifer sites where U biogeochemistry has been studied that are often electron donor poor (Andersen et al., 2003). Within the trenches, the radionuclide inventory is dominated by long lived ^{238}U and ^{234}U (half lives of 4.47×10^9 a and 2.46×10^5 a, respectively); these are significant radionuclides in terms of their mass and activity over long (250–10,000 a) and very long (>10,000 a) time scales (BNFL, 2002). Uranium is present as $U(VI)_{(aq)}$ under oxic conditions and at circumneutral pH, forms anionic carbonate complexes that are relatively mobile in groundwater (Clark et al., 1995). By contrast, U(IV) predominates under reducing conditions and is less soluble than U(VI) under pH conditions usually encountered in environmental systems U(IV) is rapidly hydrolysed and precipitates as uraninite ($UO_{2(s)}$) (Rai et al., 2003). However, under some circumstances U(IV) can form stable organic complexes, thereby increasing its solubility and mobility. For example, the complexation and solubilisation of U(IV) with the citrate ion at circumneutral pH has been reported (Macaskie, 1991; Robinson et al., 1995; Ganesh et al., 1999; Haas and Northup, 2004). Additional organic complexants such as EDTA and NTA are also found as contaminants at nuclear sites and may affect the solubility of reduced radionuclide species (Haas and Northup, 2004; Lloyd and Renshaw, 2005; Ruggiero et al., 2005).

The biogeochemical conditions within the near-field of a LLW repository will influence U solubility and migration over time. Microbially mediated $U(VI)_{aq}$ reduction in contaminated environments by indigenous microbial populations has been studied in simulations (Abdelouas et al., 1998, 2000; Fredrickson et al., 2000; Suzuki et al., 2003), and recent *in situ* studies have shown $U(VI)_{aq}$ reduction in aquifers can be stimulated by the addition of an electron donor such as acetate (Finneran et al., 2002; Senko et al., 2002; Andersen et al., 2003). Whilst these studies demonstrate microbial U(VI) removal from solution in a range of environments, little work has been done to understand the biogeochemical processes that occur in LLW repositories. As a sub-surface, cellulose-rich environment containing heterogeneous wastes, the near-field of the Drigg repository is expected to be influenced by microbially mediated redox processes (Humphreys et al., 1997; BNFL, 2002). Microorgansisms couple the oxidation of electron donors (organic substrates or H_2) to the reduction of different terminal electron acceptors (TEAs). The classic model of these processes is that a succession of microbially mediated terminal electron accepting processes (TEAPs) occurs in the order NO_3^-, Fe(III) and SO_4^{2-} reduction and methanogenesis (Reeburgh, 1983). However, the evolution of these processes in heterogeneous LLW and their potential effect on radionuclide solubility remain unclear, and understanding the biogeochemical processes that occur in the near-field environment is integral to considering the eventual fate of radionuclides in low-level wastes.

In this study, anaerobic batch culture experiments that were a simplified analogue of the Drigg LLW repository environment, were prepared. A synthetic leachate which reflected the composition of Drigg trench leachate (Humphreys et al., 1997; Ian Beadle, Nexia Solutions, Personal Communication, 2002) was prepared. The synthetic leachate contained a range of fermentation end products, but the main electron donor was acetate which has been measured on site (Humphreys et al., 1997) and the synthetic leachate was amended with inorganic constituents and a range of electron acceptors which are found in the low-level waste (i.e. NO_3^-, Fe(III), U(VI), SO_4^{2-}). The authors chose to chelate the Fe(III) with citrate in the synthetic leachate as citrate is a commonly reported organic co-contaminant at nuclear

facilities (Francis et al., 1992). The batch cultures were then inoculated with an indigenous microbial culture from the Drigg site, and the biogeochemical evolution of the batch experiments was monitored with time. Results are presented of geochemical, molecular biological and mineralogical analysis, plus geochemical modelling of these experimental systems, to provide an insight into the development of the biogeochemistry and fate of U(VI) in a near surface LLW repository.

2. Methodology

2.1. Sample collection and batch culture preparation

Trench slurry samples were collected from 4 different *in situ* stand pipes into sterile, HDPE sample pots at the Drigg site in September 2002 and immediately transported to the laboratory. The filled tubes containing the slurry were then sealed and stored at 4 °C, close to the average annual temperature on site of 10 °C (BNFL, 2002), prior to culture preparation which occurred within 2 weeks of sampling. Homogenised trench slurry (1 g) was used to create microbial enrichment cultures (75 ml) using a Drigg synthetic leachate/Fe(III)-medium adjusted to pH 7 (Table 1). The recipe for the Drigg synthetic leachate was provided by BNFL, and contained a range of inorganic and organic constituents typically found in the Drigg site leachate (Ian Beadle, Nexia Solutions, Personal Communication, 2002). The medium was prepared by addition of inorganic and organic constituents apart from $NaHCO_3$ and Fe(III)-citrate to 800 ml of 18 MΩ deionised water which was degassed with N_2, quickly adjusted to pH 6.6, and the $NaHCO_3$ added. This was then transferred to the anaerobic cabinet, Fe(III)-citrate added, the pH adjusted to 7.0, made up to 1 l, filter sterilised into 50 ml sterile serum bottles and capped. Final concentrations of terminal electron acceptors in the enrichment medium were 0.8 mM NO_3^-, 10.0 mM Fe(III) and 2.0 mM SO_4^{2-}. Acetate, the main electron donor, was an order of magnitude in excess in terms of its total electron donor capacity (ca. 440 mM) compared to the total electron accepting capacity for the terminal electron acceptors (NO_3, Fe(III) and SO_4^{2-}; ca. 30 mM) in the synthetic leachate (Table 1). Enrichment cultures were incubated in the dark at 21 °C and stocks were maintained by transferring a 10% inoculum of enrichment culture to fresh medium on a strict weekly basis, and the Drigg inoculum was sub-cultured for at least 4 weeks before experimental work began. For inoculation experiments, cells were harvested under N_2 with centrifugation for 10 min at 8000 rpm, washed twice in 50 ml of anaerobic 30 mM HCO_3 buffer with repelleting by centrifugation between washes before finally being resuspended in 0.5 mM anaerobic HCO_3 buffer (pH 6.8) under N_2.

Table 1
Key constituents of the Drigg enrichment culture media

Constituent	[a]Concentration in enrichment culture (mM)
$NaHCO_3$	64
NH_4Cl	18
KNO_3	0.8
$(NH_4)_2HPO_4$	7.6
K_2SO_4	2.0
[b]Fe(III)citrate	10
$MgCl_2 \cdot 6H_2O$	6
$CaCl_2 \cdot H_2O$	28
NaCl	44
Acetic acid	55
Propionic acid	0.5
Butyric acid	0.8
Iso-butyric acid	1.0
Iso-valeric acid	0.4
Ethanol	0.1

[a] A 10-fold dilution of this enrichment media was used in all incubation experiments.

[b] Citrate was chosen as a complexant for Fe(III) as it is expected to be present in low-level wastes (Francis et al., 1992).

A 10-fold dilution of the medium described in Table 1 was used in experiments as the standard synthetic leachate and had 5.5 mM acetate as the key electron donor and 0.08 mM NO_3^-, 1.0 mM Fe(III) and 0.2 mM SO_4^{2-} present as terminal electron acceptors. For standard experiments all manipulations were performed using aseptic technique and 25 ml of synthetic leachate was sub-aliquoted into 30 ml serum bottles in an anaerobic cabinet (Coy Laboratory Products, Michigan, USA), crimped with butyl rubber stoppers (Bellco, Vineland, NJ, USA) and autoclaved (under standard conditions of 121 °C, 15 psi for 20 min). Bottles were then inoculated with 2.5 ml of the harvested, washed Drigg enrichment culture cells. Heat killed controls containing non-active biomass were established by inoculating the sterile medium with enrichment culture cells and then immediately autoclaving.

2.2. Experimental programme

Three key experimental systems were studied: (1) Drigg culture without uranyl present; (2) Drigg culture with uranyl present; and (3) Drigg culture with

uranyl present but with dissimilatory SO_4^{2-} reduction inhibited by addition of Na molybdate to a final concentration of 20 mM. Appropriate sterile and electron donor-free controls were also run. In addition to the key anaerobic incubations described above, cell suspensions were also incubated under oxic conditions and in the presence of $U(VI)_{(aq)}$ to quantify sorption of uranyl onto cell surfaces. For cultures containing $U(VI)_{aq}$, filter sterilised (<0.2 μm) aliquots of a UO_2Cl_2 stock solution were added to a final concentration of 126 μM (corresponding to ca. 400 Bq kg^{-1} ^{238}U, the lower legal limit for alpha activity in low-level wastes). All bottles were incubated under anaerobic conditions at 21 °C for 120 h and sacrificially sampled over time. Five replicates were sampled at each time point to assess reproducibility and the mean $\pm 1\sigma$ of these replicates is reported.

2.3. Analytical techniques

Nitrate, $Fe(II)_{(aq)}$, total aqueous Fe ($Fe(II) + Fe(III)_{(aq)}$), and total Fe ($Fe_{(s)} + Fe_{(aq)}$) and sulfide were analysed using standard spectroscopy measurements (Cline, 1969; Lovley and Phillips, 1986; Viollier et al., 2000; Harris and Mortimer, 2002). Uranium $(VI)_{(aq)}$ was determined using the bromo-PADAP method (Johnson and Florence, 1971) and total $U_{(aq)}$ ($U(VI)_{(aq)} + U(IV)_{(aq)}$) in samples was determined by oxidising filtered samples with HNO_3 to a final concentration of ~0.1 M for at least 24 h (Ganesh et al., 1999) and then analysing using the bromo-PADAP method. Total uranium (aq + s) in the samples was determined by bromo-PADAP analysis on unfiltered, HNO_3 oxidised samples. Nitrate and SO_4^{2-} were determined by ion chromatography using a Dionex DX100 with AS4 or AS14 analytical column. Acetate was determined by ion exclusion chromatography using a Dionex AS1 column. Solids that had precipitated at the end of incubations were analysed using a combination of XRD on a Phillips PW1050 and scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) on a Cameca SX-50. Prior to XRD, the solid material was centrifuged in an anaerobic cabinet (15,000 rpm 30 min), washed with deoxygenated, deionised water, and then a subsample was mounted in an anaerobic sample holder. A sample of the same material was transferred to a Cu grid in an anaerobic cabinet, dried overnight and analysed using SEM-EDX.

2.4. Geochemical modelling

Two systems, representative of the Drigg and U-Drigg cultures, were modelled using PHREEQC V2 equilibrium geochemical modelling software and the MINTEQA2 database. Additional U(IV)-citrate thermodynamic data were also included in the database (Grenthe, 1992). The geochemical speciation of key components of the batch culture experiments was modelled under progressively anoxic conditions using pε values calculated from experimentally derived E_h measurements and pertinent modelling results are described in the discussion.

2.5. Microbial activity

The ATPLite kit (PerkinElmer, UK) was used to determine ATP in incubations because ATP acts as a proxy for biomass and biomass activity (Brookes and Ocio, 1989). Unfiltered samples (100 μl) from both inoculated and sterile systems were lysed using 50 μl of 1 M NaOH, shaken, and treated with 50 μl of luciferase/luciferin for 5 min. Light output was determined on a Packard Topcount NXT luminesence counter.

2.6. Microbial ecology

Changes in the diversity of the microbial community in selected experiments were determined by denaturing gradient gel electrophoresis (DGGE) to provide a ''genetic fingerprint'' of the microbial population in the samples (Nakatsu et al., 2000). Enrichment cultures were boiled (100 °C, 5 min) to lyse any microbial cells and liberate intracellular nucleic acids. A fragment of the 16S rRNA gene (approximately 200 b.p.) was amplified by PCR from samples using the broad specificity primers GC338f and 530r (van der Gast et al., 2001). The presence of amplification products was verified by electrophoresis in 2% agarose gels stained with ethidium bromide. DGGE was then performed utilising 20 μl of the amplified product, loaded onto a 10% (w/v) polyacrylamide gel with a 40–60% denaturing gradient in 0.5% Tris Acetate EDTA (TAE) buffer. Electrophoresis was undertaken for 16 h at a controlled temperature of 60 °C and constant voltage of 100 V. Gels were stained for 20 min in 0.5% TAE buffer containing 2000 mg l^{-1} Syber Gold (Molecular Probes Inc., Oregon, USA) and imaged under short-wave UV light.

In order to identify the dominant microorganisms in the samples, a fragment of the 16S rRNA gene (approximately 520 b.p.) was amplified by PCR from samples using the broad specificity primers 8f and 519r (Islam et al., 2004). PCR products were purified using a QIAQuick purification kit (Qiagen) and ligated directly into the cloning vector pCR 2.1 (Invitrogen) before transformation into *Escherichia coli* TOP 10 competent cells. White transformants that grew on LB agar containing ampicillin (100 μg ml^{-1}) were screened for an insert using PCR and products purified with a QIAQuick kit. Clones were separated into operational taxonomic units (OTUs) based upon the similarity of restriction fragment length polymorphism (RFLP) profiles, using the restriction enzymes *Sau*3A and *Msp*I. The nucleotide sequences of each OTU were determined using the dideoxynucleotide method and sequences analysed against the NCBI (USA) BLAST database, with matching to known 16S rDNA gene sequences.

3. Results and discussion

3.1. The Drigg culture without uranyl present

3.1.1. Geochemistry

Sterile controls showed no changes in terminal electron acceptors (open squares in Fig. 1a–c). In contrast, Drigg synthetic trench leachate amended with the Drigg microbial consortium showed removal of NO_3^- from solution, followed by ingrowth of Fe(II) into solution, and then ingrowth of Fe(II) to solids concomitant with the removal of SO_4^{2-} from solution (Fig. 1a–c). This demonstrates that progressive anoxia developed in the order NO_3^-, Fe(III) and SO_4^{2-} reduction, confirming that microorganisms capable of a range of terminal electron accepting processes were active in the Drigg culture. Nitrate reduction, as indicated by removal of NO_3^- from solution, was completed within the first 12 h. Iron (III) reduction, as indicated by ingrowth of $Fe(II)_{aq}$ and $Fe(II)_s$, followed NO_3^- reduction. Iron $(II)_{aq}$ concentrations peaked at 48 h, coincident with the onset of SO_4^{2-} reduction and the ingrowth of a black, X-ray amorphous solid. SEM-EDX analysis of the black solid taken at the end time point confirmed the presence of both Fe and S, and it liberated a "rotten eggs" smell on addition of 1.0 M HCl. This suggested that some of the material was amorphous Fe sulfide and at 120 h the molar ratio of Fe:S in the solid was 2:1.

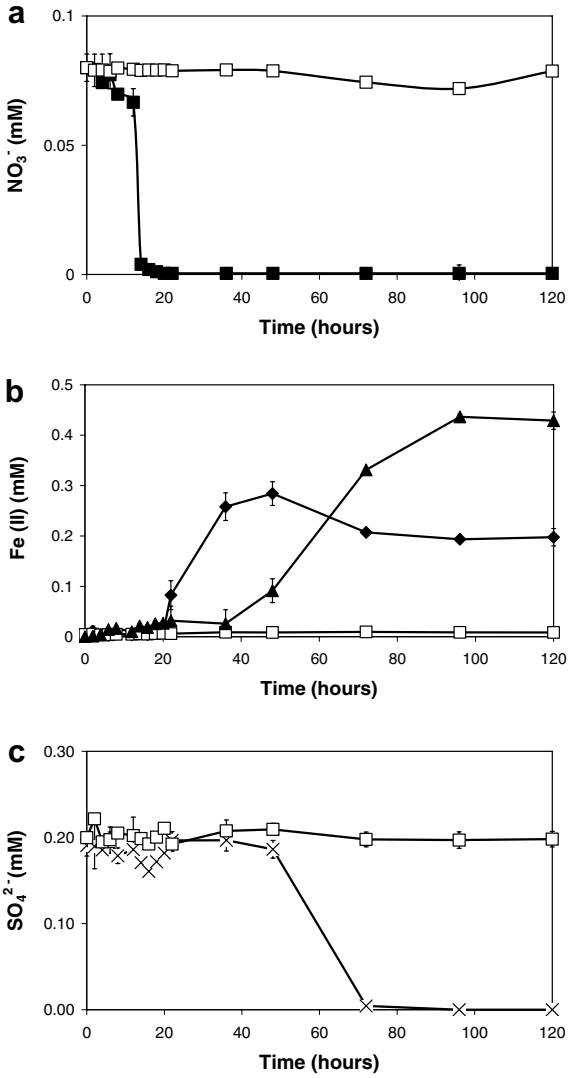

Fig. 1. Time series data showing the changes in redox indicator species in incubations with Drigg synthetic leachate: (a) NO_3^- concentrations in active experiments (■) and sterile controls (□); (b) Fe(II) concentrations in solution in active experiments (◆) and sterile controls (□) and Fe(II) ingrowth to solid phases (▲); and (c) SO_4^{2-} concentrations in active experiments (×) and sterile controls (□). Error bars are 1σ of 5 replicates (where not shown, errors are within size of symbol).

Sulfate was completely removed from solution by 96 h, but active Fe(II) ingrowth to solids continued thereafter (Fig. 1b) again suggesting that another Fe(II) mineral was forming in the microcosms. Analysis of ATP (data not shown) showed an initial small peak in the ATP concentration between 8 and 9 h during active NO_3^--removal, then a decline followed by an increase to a maximum between 48 and 72 h and a final decline to 120 h. This suggests that

active biomass fluctuated throughout the experiment with detectable growth of biomass during NO_3^- reduction, and more significantly during the main period of Fe(III) and SO_4^{2-} reduction. The acetate concentration remained constant in the controls, but acetate was consumed in the inoculated systems (data not shown). At the final point in the experiment there was an electron balance between production of electrons from oxidation of acetate and removal of electrons by NO_3^-, Fe(III) and SO_4^{2-} reduction (Table 2).

3.1.2. Microbial ecology

Analysis of the microbial diversity in the samples using DGGE showed that there was little temporal variation in the microbial community in the samples with no U present over the relatively short (120 h) time course of the experiments. A more detailed analysis of the final time-point sample was undertaken *via* assessment of the genetic diversity of cloned 16S rRNA gene fragments. Of the 29 clones analysed, only 8 gave distinct RFLP profiles, suggesting that as expected a relatively simple microbial community had developed within these enrichment culture based experiments (Fig. 2a). Close relatives of known NO_3^--reducing bacteria *Azospira oryzae* (98% similarity, 523 out of 525 base matches) were detected in the clone library. In addition, a close relative to *Quadricoccus australiansis* (98% similarity, 508 out of 514 base matches) was also present and is phylogenetically placed between *Ferribacterium* sp. and *Dechloromonas* sp., suggesting a potential capability for Fe(III) reduction and also U(VI) reduction. Additionally, close relatives of *Brevundimonas diminuta* (98% similarity, 467 out of 472 base matches) and *Trichococcus pasteurii* (99% similarity, 526 out of 531 base matches) were also detected in the clone libraries. Interestingly, there were no close relatives of known SO_4^{2-}-reducing bacteria in the clone library, although as discussed above, SO_4^{2-} was a significant component of the synthetic leachate in terms of its electron accepting equivalents. Overall these results suggest a surprisingly novel microbial population in the enrichment cultures without U present, with a relative absence of commonly occurring Fe(III)-reducing bacteria such as *Geobacter* sp., which have previously been reported to be dominant in some heavy metal and U-contaminated sub-surface aquifer environments (Andersen et al., 2003; Islam et al., 2004). However, members of *Rhodocyclaceae* sp. which include *Azospira* sp., *Quadricoccus* sp. and *Dechloromonas* sp. were detected in the clone libraries, and close relatives of these microorganisms have previously been detected in U(VI)-contaminated mines (Suzuki et al., 2003).

3.2. Drigg culture with U present

3.2.1. Geochemistry

The second experiment focussed on examining the effects of addition of U(VI) to the experimental system described in Section 1. Microcosms were set up with synthetic leachate and with addition of 126 μM UO_2Cl_2 and the results are shown in Fig. 3a–d. Nitrate was completely removed from

Table 2
Electron balance calculations for experiments with: (a) the Drigg culture without U(VI) present and (b) the Drigg Culture with U(VI) present

Oxidative processes	Reductive processes
(a)	
Acetate $\rightarrow HCO_3^- + H_2O + 8e^-$ (0.45 ± 0.1 mM)	$NO_3^- + 5e^- \rightarrow N_2$ (0.08 mM)
	$Fe(III) + e^- \rightarrow Fe(II)aq$ (0.20 mM)
	$Fe(III) + e^- \rightarrow Fe(II)s$ (0.29 mM)
	$SO_4^{2-} + 8e^- \rightarrow HS^-$ (0.20 mM)
Total electrons produced = 3.60 ± 0.8 mM	Total electrons consumed = 2.49 mM
(b)	
Acetate $\rightarrow HCO_3^- + H_2O + 8e^-$ (0.43 ± 0.07 mM)	$NO_3^- + 5e^- \rightarrow N_2$ (0.08 mM)
	$Fe(III) + e^- \rightarrow Fe(II)aq$ (0.50 mM)
	$Fe(III) + e^- \rightarrow Fe(II)s$ (0.30 mM)
	$U(VI) + 2\,e^- \rightarrow U(IV)$ (0.14 mM)
	$SO_4^{2-} + 8e^- \rightarrow HS^-$ (0.20 mM)
Total electrons produced = 3.44 ± 0.56 mM	Total electrons consumed = 3.08 mM

Balances were calculated for the 120 h time point in experiments, assuming reduction of NO_3^- to N_2, Fe(III) to Fe(II), SO_4^{2-} to HS^- and where relevant U(VI) to U(IV), and oxidation of acetate to HCO_3^- and H_2O.

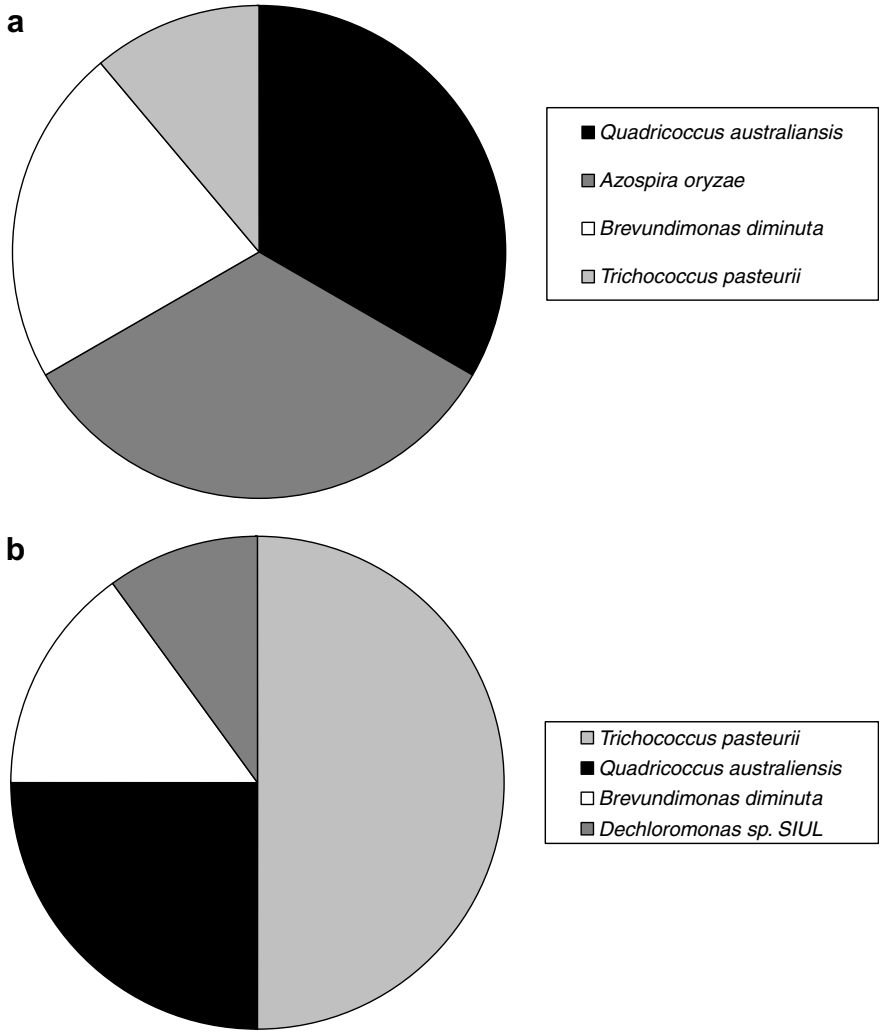

Fig. 2. Microbial community analysis for end point samples from: (a) Drigg synthetic leachate and (b) Drigg synthetic leachate and 0.126 mM U(VI).

the microbially active experiment (only) after 20 h, reflecting a pattern of NO_3^- reduction identical to the first experiment where U was not present. Removal of 13% of U(VI) occurred over the first 12 h of the experiment in sterile controls, acetate depleted controls, and in active experiments but no removal of U(VI) was seen in this period of NO_3^- reduction. This initial removal of U(VI) was attributed to sorption to biomass as both experimental results and geochemical modelling suggested that U(VI)-mineral phases were undersaturated in the experimental medium such that precipitation was not expected in the experiments. In addition, removal of 10–20% of initial U(VI) also occurred in acetate depleted and sterile controls with cell biomass present. No further U(VI)-removal from solution occurred during NO_3^- reduction, which is similar to observations in experiments examining U(VI) biogeochemistry in batch experiments and field experiments with aquifer sediments (Abdelouas et al., 1998, 2000; Istok et al., 2004).

In the inoculated system, the concentration of total U in solution remained constant throughout the experiment (following initial sorption of U(VI) to biomass) but $U(VI)_{aq}$ decreased. This suggests that U(VI) reduction led to a soluble U(IV) species and geochemical modelling predicted that U(IV)–citrate complexes formed in these experiments.

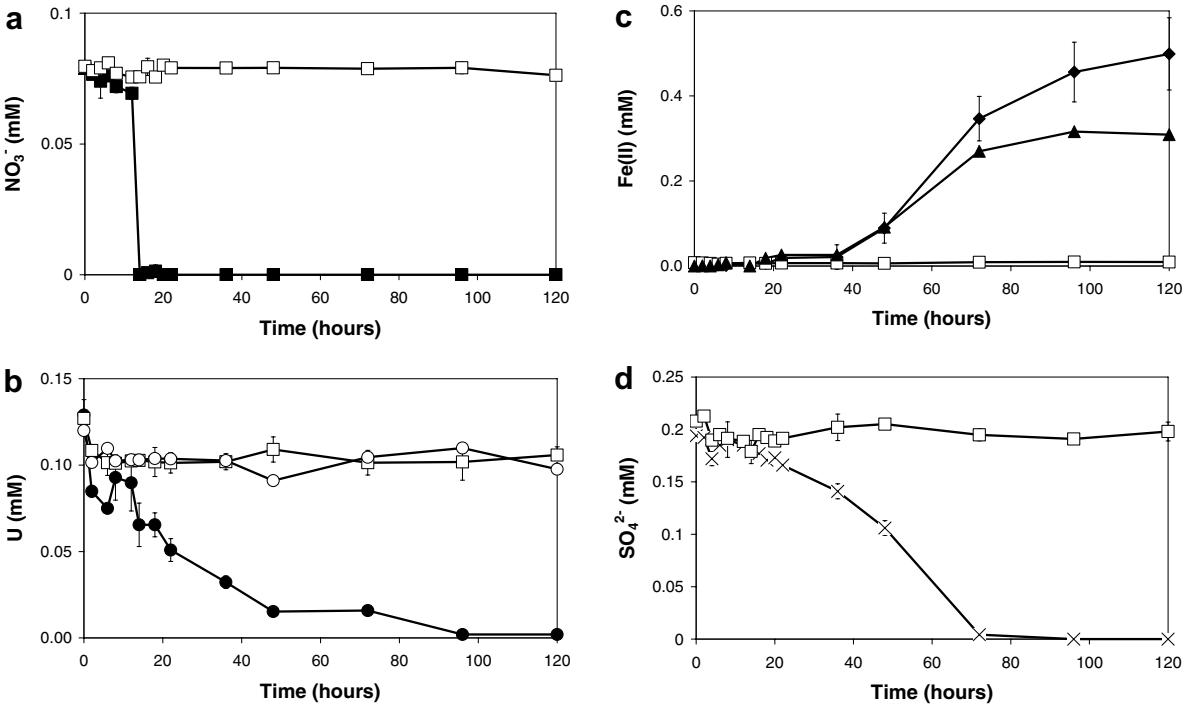

Fig. 3. Time series data showing the changes in redox indicator species in incubations with Drigg synthetic leachate and 0.126 mM U(VI): (a) NO_3^- concentrations in active experiments (■) and sterile controls (□); (b) $U(VI)_{aq}$ concentrations in active experiments (●), sterile controls (□), and U(VI) + (IV) concentrations in active experiments (○); (c) Fe(II) concentrations in solution in active experiments (◆) and sterile controls (□) and Fe(II) ingrowth to solid phases (▲); and (d) SO_4^{2-} concentrations in active experiments (×) and sterile controls (□). Error bars are 1σ of 5 replicates (where not shown, errors are within size of symbol).

Measurable U(VI) reduction started between 12 and 18 h, ~88% U(VI) was reduced by 72 h, and slow U(VI) removal continued to 120 h. In the inoculated system, Fe(II) in solution was measurable at 22 h, however, it was just above background, and was lower at the comparable time in the system with no U(VI) present (Fig. 1b and Fig. 2c). Substantial ingrowth of $Fe(II)_{aq}$ only occurred from 36 h onwards, and then $Fe(II)_{aq}$ increased to a maximum of 0.50 mM by 120 h. This was double the Fe(II) concentration seen at the final time point in the experiment with no U(VI), suggesting that Fe(III) reduction is more active at the end of the experiment than in the system with no U present. Ingrowth of Fe(II) into a solid phase was observed from 24 h and continued until the end of the experiment. A black precipitate similar to that observed in the previous experiment appeared from 48 h and again SEM-EDX analysis of this sample confirmed the precipitate contained Fe and S confirming the presence of amorphous FeS. At 120 h, the molar ratio of Fe:S in the solid was 1.5 suggesting that another, unidentified Fe(II) phase contributed to the precipitate seen in the experiment but that the partitioning of Fe(II) to solution was higher in the presence of U. There was no U detected in the solid sample, in agreement with the mass balance showing the majority of U was present in solution. This suggests that Fe(III) reduction was occurring from 22 h, but that significant Fe(III) reduction only occurred later than in experiments with no U(VI) present. Sulfate concentrations decreased from as early as 12 h, however, significant SO_4^{2-}-removal was only measurable between 22 and 36 h. This is different from the pattern seen in the experiment without U(VI) where SO_4^{2-} reduction began between 48 and 72 h. Finally, ATP and acetate profiles were similar to experiments without U present indicating U(VI) did not have a toxic effect on the system. At the final point in the experiments, there was an electron balance between production of electrons from oxidation of acetate and removal of electrons by NO_3^-, Fe(III), U(VI) and SO_4^{2-} reduction (Table 2). Overall, the pattern of microbial reduction in these experiments was altered compared to systems without U present, with

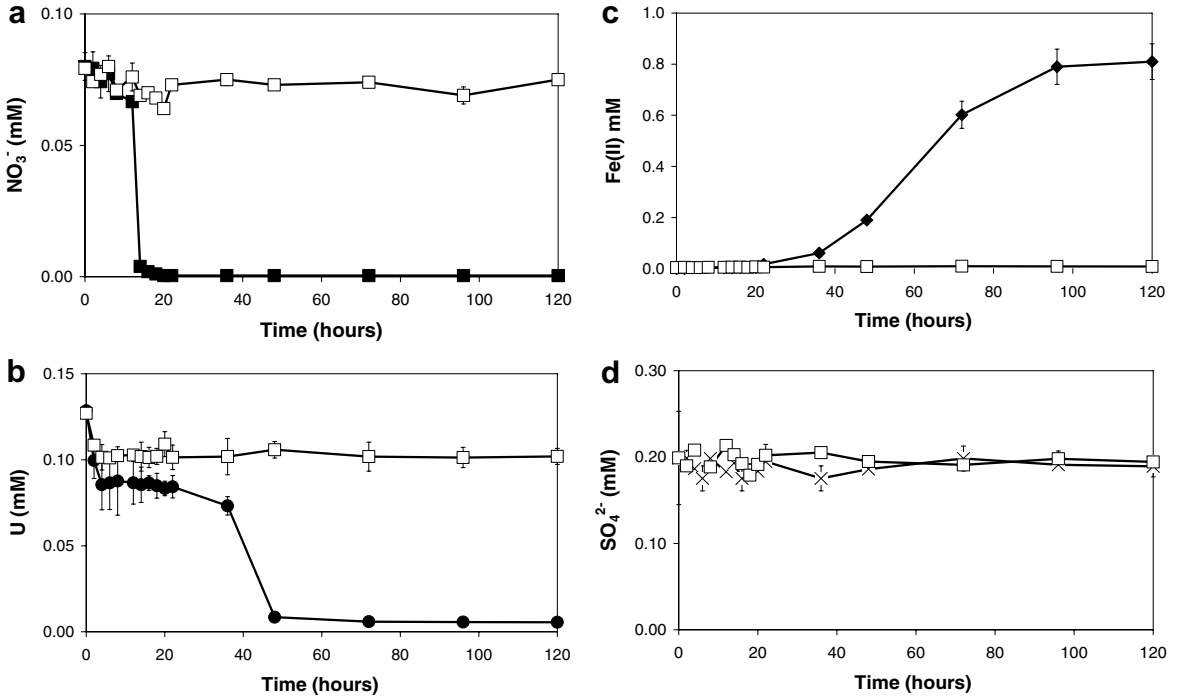

Fig. 4. Time series data showing the changes in redox indicator species in incubations with Drigg synthetic leachate and 0.126 mM U(VI) with dissimilatory SO_4^{2-} reduction inhibited: (a) NO_3^- concentrations in active experiments (■) and sterile controls (□); (b) $U(VI)_{aq}$ concentrations in active experiments (●) and sterile controls (□); (c) Fe(II) concentrations in solution in active experiments (◆) and sterile controls (□); and (d) SO_4^{2-} concentrations in active experiments (×) and sterile controls (□). Error bars are 1σ of 5 replicates (where not shown, errors are within size of symbol).

U(VI)-removal starting after NO_3^- reduction, and occurring at the same time as both Fe(III) and SO_4^{2-} reduction. Sulfate reduction dominated in the mid-portion of the experiment, and the majority of Fe(III) reduction occurred in the latter stages of the experiment.

3.2.2. Microbial ecology

Characterisation of the microbial population by DGGE profiling during the time course of the experiments showed similar profiles compared to U-free results until 72 h (data not shown). At the last sampling point, one band in the DGGE profile became more prominent, suggesting a measurable alteration in the microbial community in these systems may have been occurring toward the end of the experiment. DGGE of the samples for final time points in both the Drigg culture experiments with no U present, and with added U(VI), showed distinct differences in the microbial abundance and diversity in the samples which was further confirmed by analysis of 16SrDNA clone libraries (Fig. 2). The PCR/RFLP characterisation using broad specificity primers on these samples revealed very similar matches to the microbes found in the U(VI)-deficient system but with an altered dominance in the experiments with U(VI) present. Here, there was an increase in *Trichococcus* sp. and *Dechloromonas* sp. and a decrease in *Azospira* sp. (Fig. 2). Again, there were no close relatives of known SO_4^{2-}-reducing bacteria in the clone library.

3.3. Drigg culture with uranyl present – sulfate reduction inhibited

To further investigate the processes occurring in the incubations, an experiment was undertaken where dissimilatory SO_4^{2-} reduction was inhibited by Na molybdate (Fig. 4a–d). In this experiment, NO_3^- remained unchanged in controls but was completely removed by microbial NO_3^- reduction in the inoculated system within 14 h. Iron(II) was not observed in sterile controls, but in the inoculated system its ingrowth to solution was similar to the system with U(VI) present but not DSR inhibited. Here, measurable Fe(II) ingrowth was observed at

22 h, with the most significant increase in Fe(II) in solution occurring between 48 and 72 h. The concentration of Fe(II) in solution in this experiment was the highest of all 3 experiments with 75–85% of the 1 mM Fe(III) in the initial experiment reduced by 120 h possibly due to reduced competition with SO_4^{2-}-reducing bacteria for electron donor in the presence of molybdate (Canfield et al., 1993). As expected, SO_4^{2-} reduction was inhibited, and SO_4^{2-} concentrations remained constant in both sterile and inoculated incubations throughout the experiment. The inhibition of SO_4^{2-} reduction meant that no Fe sulphide phases could form in these experiments and that Fe(II) was more extensively partitioned to solution than in other experiments. In fact, a trace amount of precipitate developed in these microcosms from 48 h onward, however, unfortunately the quantity of material was too small to harvest for characterisation. The U(VI) reduction, as indicated by removal of $U(VI)_{aq}$ in inoculated microcosms only, was similar to the previous experiment with U(VI) present, and was essentially complete by 72 h. This suggests that the precipitation of Fe sulfide phases seen in SO_4^{2-}-reducing microcosms is not a key control on reduction of U(VI) in these incubations and in combination with the microbial ecology results, indicates that microbially mediated U(VI) reduction is likely dominated by Fe(III)-reducing microorganisms.

3.4. Synthesis of results

In all experiments, terminal electron accepting processes proceeded robustly and typical microcosm experiments were complete in 120 h. The microcosm experiments described here are designed to be representative of conditions within Drigg. However, the rate of development of microbially mediated anoxia in microcosms (where conditions are optimised for microbial growth) is unlikely to be representative of the Drigg site which is a heterogeneous mixed waste disposal site. Nevertheless, these experiments clearly demonstrate that the Drigg LLW facility contains microorganisms capable of a range of terminal electron accepting processes under microcosm conditions. Nitrate reduction occurred at a similar rate in all 3 systems and was largely complete within 14 h, suggesting that NO_3^--reducers were unaffected by U(VI) or molybdate additions. Indeed, denitrification has been reported extensively in U-contaminated environments and in the experiments, it occurred prior to significant U(VI) reduction in agreement with past work at contaminated aquifer sites (Abdelouas et al., 1998, 2000; Istok et al., 2004).

Iron(III) reduction, as indicated by Fe(II) ingrowth in solid and solution phases occurred in all 3 experiments, although there were differences in the fate of Fe in the different experiments. In systems with and without U a black Fe sulfide mineral precipitated and scavenged Fe(II) from solution. Interestingly, U was not scavenged to the sulfide precipitate in the experiment with U present, suggesting that abiotic reduction to Fe(II) minerals including sulfides does not dominate in these systems. Overall, the extent of sulfide mineral formation was reduced in the system with U present as Fe(II) solution concentrations were increased compared to the system without U. It is unclear why the partitioning of reduced Fe was different between these two systems. Finally, in the experiment where SO_4^{2-} reduction was inhibited, sulfide formation did not occur, and thus the vast majority of reduced Fe(II) was partitioned to the solution phase with approximately 80% of the Fe(III) available at the start of the experiment reduced by 120 h.

In the Drigg culture experiment, measurable U(VI) reduction occurred between 12 and 18 h, prior to extensive Fe(III) or SO_4^{2-} reduction. The dominant period of U(VI) reduction occurred between 18 and 48 h at the same time as initial SO_4^{2-} reduction and prior to significant Fe(III) reduction indicated by Fe(II)-ingrowth into solution. Indeed, substantial Fe(III) reduction only started between 36 and 48 h. This confirmed that U(VI), Fe(III) and SO_4^{2-} reduction were occurring concomitantly in these experiments and that the addition of U(VI) to the system affected the gross order of terminal electron accepting processes. Finally, measurable U(VI) reduction occurred between 22 and 36 h in the system where SO_4^{2-} reduction was inhibited by molybdate.

The similarity of U(VI) reduction profiles between experiments with and without SO_4^{2-} reduction inhibited suggests that SO_4^{2-}-reducing microorgansisms are not dominant in controlling U(VI)-reduction in these systems and that abiotic reduction of U(VI) by sulfide solid phases is unlikely to dominate in these experiments, presumably due to the preferential formation of soluble U(IV)–citrate complexes as suggested by past workers (Ganesh et al., 1997; Robinson et al., 1995; Haas and Northup, 2004). Additionally, the observation that U(VI) reduction was occurring prior to significant

Fe(II) ingrowth into either solution or solid in the Drigg experiment and to solution in the molybdate inhibited experiment suggests that abiotic reduction of U(VI) *via* reaction with Fe(II) is not significant in these systems. Indeed, $Fe(II)_{aq}$ is considered to be an ineffectual reductant for U(VI) under environmentally relevant conditions (Liger et al., 1999). This evidence, taken as a whole, suggests that U(VI) reduction in these complex systems is mediated by Fe-reducing bacteria *via* enzymatic reduction.

Interestingly, microbial characterisation showed no close relatives to known SO_4^{2-}-reducing microorganisms but detected a range of microorganisms that had the potential to reduce metals including U(VI) with close relatives of the *Rhodocyclus-Azoarcus* subgroup of the β-proteobacteria found in cultures with and without U(VI) added, as well as *Quadricoccus* spp., which is phylogenetically placed between *Ferribacterium* and *Dechloromonas* suggesting possible metal-reducing capability. Cultures with U(VI) present had elevated proportions of microbes closely related to *Trichococcus pasteurii* and *Dechloromonas* spp. Many species in the *Rhodocyclus-Azoarcus* group are capable of facultative anaerobic growth and are known to favour denitrification coupled to degradation of aromatic compounds (Song et al., 2001; Waller et al., 2004). Furthermore, relatives of this group have recently been detected in microaerophillic U-contaminated environments (Suzuki et al., 2003) and have been found in significant numbers at a radioactive waste facility (Nazina et al., 2004). Therefore, the microorganisms observed in the Drigg enrichment culture are novel compared to many studies focussed on contaminated aquifer systems, but fully expected in engineered U-contaminated environments.

4. Conclusions

In the experiments, U(VI) was completely reduced to U(IV) by indigenous microorganisms from a LLW repository demonstrating the potential for these reactions to occur within the Drigg waste site. No $U(IV)O_2$ precipitation occurred in the synthetic leachate presumably due to complexation of $U(IV)_{(aq)}$ with citrate. In the Drigg LLW facility microbial U(VI) reduction would be expected to lead to precipitation of reduced U as $UO_{2(s)}$ in the absence of strong complexants such as citrate. However, organic complexants such as citrate, EDTA and NTA may be present in some radioactive wastes due to their use in the nuclear fuel cycle and may contribute to enhancing U(IV) solubility within the waste disposal facility. There were significant differences in the microbial communities in systems with and without U(VI) present. In addition, the biogeochemistry of the system with U(VI) behaved differently to the system without U as SO_4^{2-} reduction occurred simultaneously with Fe(III) and U(VI) reduction. The consortium of microorganisms isolated from Drigg was capable of NO_3^-, $U(VI)_{(aq)}$, Fe(III) and SO_4^{2-} reduction. Microbial community analysis showed that the microorganisms present are typical for U-contaminated, engineered environments. Overall, these experiments confirm that microbially mediated anoxia is capable of occurring within the Drigg trench environment, and that U(VI) reduction and removal to UO_2 is likely to occur within the trenches in the absence of strong complexants such as citrate.

Acknowledgements

This work was supported by a case studentship NER/S/M/2000/03973 for JRF from NERC and BNFL. We thank Paul Humphreys for his help in initiating this project and Miranda Keith-Roach for valuable discussions prior to submission. We appreciate the helpful comments of Dr. J.R. Haas and another anonymous reviewer.

References

Abdelouas, A., Lu, Y., Lutze, W., Nutall, H.E., 1998. Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated groundwater. J. Contam. Hydrol. 35, 217–233.

Abdelouas, A., Lutze, W., Gong, W., Nuttall, H.E., Strietelmeier, B.A., Travis, B.J., 2000. Biological reduction of uranium in groundwater and subsurface soil. Sci. Total Environ. 250, 21–35.

Andersen, R.T., Vrionis, H.A., Ortiz-Bernad, I., Resch, C.T., Long, P.E., Dayvault, R., Karp, K., Marutzky, S., Metzler, D.R., Peacock, A., White, D.C., Lowe, M., Lovley, D.R., 2003. Simulating the in-situ activity of *Geobacter* sp. to remove uranium from the groundwater of a uranium contaminated aquifer. Appl. Environ. Microbiol. 69, 5884–5891.

BNFL, 2002. Drigg Post Closure Safety Case – Overview Report. British Nuclear Fuels Limited.

Brookes, P.C., Ocio, J.A., 1989. The use of ATP measurements in soil microbial biomass studies. In: Stanley, P.E., McCarthy, R.B., Smither, R. (Eds.), ATP Luminescence: Rapid Methods in Microbiology. Blackwell Scientific.

Canfield, D.E., Thamdrup, B., Hansen, J.W., 1993. The anaerobic degradation of organic matter in Danish coastal

sediments: iron reduction, manganese reduction, and sulphate reduction. Geochim. Cosmochim. Acta 57, 3867–3883.

Clark, D.L., Hobart, D.E., Neu, M.P., 1995. Actinide carbonate complexes and their importance in actinide environmental chemistry. Chem. Rev. 95, 25–48.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanog. 14, 454–458.

Finneran, K.T., Anderson, R.T., Nevin, K.T., Lovley, D.R., 2002. Potential for bioremediation of uranium contaminated aquifers with microbial U(VI) reduction. Soil Sed. Contam. 11, 339–357.

Francis, A.J., Dodge, C.J., Gillow, J.B., 1992. Biodegradation of citrate metal complexes and implications for toxic metal mobility. Nature 356, 140–142.

Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Duff, M.C., Gorby, Y.A., Li, S.W., Krupka, K.M., 2000. Reduction of U(VI) in goethite (a-FeOOH) suspensions by a dissimilatory metal-reducing bacterium. Geochim. Cosmochim. Acta 64, 3085–3098.

Ganesh, R., Robinson, K.G., Chu, L.L., 1999. Reductive precipitation of U(VI) by *Desulfovibrio desulfuricans*: evaluation of co-contaminant effects and selective removal. Water Res. 33, 3447–3458.

Ganesh, R., Robinson, K.G., Reed, G.D., Sayler, G.S., 1997. Reduction of hexavalent uranium from organic complexes by sulfate and iron reducing bacteria. Appl. Environ. Microbiol. 63, 4385–4391.

Grenthe, I., 1992. Chemical thermodynamics of uranium. OECD/NEA. Available from: <http://www.nea.fr/html/dbtdb/>.

Haas, J.R., Northup, A., 2004. Effects of aqueous complexation on reductive precipitation of uranium by *Shewanella putrefaciens*. Geochem. Trans. 5, 41–48.

Harris, S.J., Mortimer, R.J.G., 2002. Determination of nitrate in small volume samples by the cadmium-copper reduction method: a manual technique with application to the interstitial waters of marine sediments. Internat. J. Environ. Anal. Chem. 82, 369–376.

Humphreys, P.N., McGarry, R., Hoffman, A., Binks, P., 1997. DRINK: a biogeochemical source term model for low-level radioactive waste disposal sites. FEMS Microbial Rev. 20, 557–571.

Islam, F.S., Gault, A.G., Boothman, C., Polya, D.A., Charnock, J.M., Chatterjee, D., Lloyd, J.R., 2004. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature 430, 68–71.

Istok, J.D., Senko, J.M., Krumholz, L.R., Watson, D., Bogle, M.A., Peacock, A., Chang, Y.J., White, D.C., 2004. In-situ bioreduction of technetium and uranium in a nitrate-contaminated aquifer. Environ. Sci. Technol. 38, 468–475.

Johnson, D.A., Florence, T.M., 1971. Spectrophotometric determination of uranium(VI) with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol. Anal. Chim. Acta 53, 73–79.

Liger, E., Cherlet, L., van Cappellen, P., 1999. Surface catalysis of U(VI) reduction by Fe(II). Geochim. Cosmochim. Acta 63, 2939–2955.

Lloyd, J.R., Renshaw, J.C., 2005. Bioremediation of radioactive waste: radionuclide microbe interactions in laboratory and field scale studies. Curr. Opin. Biotechnol. 16, 254–260.

Lovley, D.R., Phillips, E.J.P., 1986. Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Appl. Environ. Microbiol. 52, 751–757.

Macaskie, L.E., 1991. The application of biotechnology to the treatment of waste produced from nuclear fuel cycle: biotechnology and bioaccumulation as a means of treating radioactive materials containing streams. Crit. Rev. Biotechnol. 11, 4–112.

Nakatsu, C., Torsvik, V., Ovreas, L., 2000. Soil community analysis using DGGE of 16s rDNA polymerase chain reaction products. Soil Sci. Soc. Am. J. 64, 1382–1388.

Nazina, T.N., Kosareva, I.M., Petrunyaka, V.V., Savushkina, M.K., Kudriavtsev, E.G., Lebedev, V.A., Ahunov, V.D., Revenko, Y.A., Khafizov, R.R., Osipov, G.A., Belyaev, S.S., Ivanov, M.V., 2004. Microbiology of formation waters from the deep repository of liquid radioactive wastes, Severnyi. FEMS Microbial Ecol. 49, 97–107.

Rai, D., Yui, M., Moore, D.A., 2003. Solubility and solubility product at 22 °C of UO_2 precipitated from aqueous U(IV) solutions. J. Sol. Chem. 32, 1–17.

Reeburgh, W.S., 1983. Rates of biogeochemical processes in anoxic sediments. Ann. Rev. Earth Planet Sci. 11, 269–298.

Robinson, K., Ganesh, G.R., Reed, G.D., 1995. Impact of organic ligands on uranium removal during anaerobic biological treatment. Water Sci. Technol. 37, 73–80.

Ruggiero, C.E., Boukhalfa, H., Forsythe, J.H., Lack, J.G., Hersman, L.E., Neu, M.P., 2005. Actinide and metal toxicity to prospective bioremediation bacteria. Environ. Microbiol. 7, 88–97.

Senko, J.M., Istok, J.D., Suflita, J.M., Krumholz, L.R., 2002. In-situ evidence for uranium immobilisation and remobilisation. Environ. Sci. Technol. 36, 1491–1496.

Song, B., Palleroni, N.J., Kerkhof, L.J., Haggblom, M.M., 2001. Characterization of halobenzoate-degrading, denitrifying *Azoarcus* and *Thauera* isolates and description of *Thauera chlorobenzoica* sp. nov.. Int. J. Syst. Evolution Microbiol. 51, 589–602.

Suzuki, Y., Kelley, S.D., Kemner, K.A., Banfield, J.F., 2003. Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. Appl. Environ. Microbiol. 69, 1337–1346.

van der Gast, C.J., Knowles, C.J., Wright, M.A., Thompson, I.P., 2001. Identification and characterisation of bacterial populations of an in-use metal-working fluid by phenotypic and genotypic methodology. Internat. Biodet. Biodeg. 47, 113–123.

Viollier, E., Inglett, P.W., Hunter, K., Roychoudhury, P., Van Cappellen, P., 2000. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Appl. Geochem. 15, 785–790.

Waller, A.S., Cox, E.E., Edwards, E.A., 2004. Perchlorate-reducing microorganisms isolated from contaminated sites. Environ. Microbiol. 6, 517–527.