



Microbially mediated reduction of Np(V) by a consortium of alkaline tolerant Fe(III)-reducing bacteria

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[Received 28 October 2014; Accepted 30 March 2015; Associate Editor: Nicholas Evans]

ABSTRACT

Neptunium-237 will be present in radioactive wastes over extended time periods due to its long half-life (2.13×10^6 years). Understanding its behaviour under conditions relevant to radioactive waste disposal is therefore of particular importance. Here, microcosm experiments were established using sediments from a legacy lime workings with high-pH conditions as an analogue of cementitious intermediate-level radioactive waste disposal. To probe the influence of Fe biogeochemistry on Np(V) in these systems, additional Fe(III) (as ferrihydrite) was added to select experiments. Biogeochemical changes were tracked in experiments with low levels of Np(V) (20 Bq ml^{-1} ; $3.3 \mu\text{M}$), whilst parallel higher concentration systems (2.5 KBq ml^{-1} ; $414 \mu\text{M}$) allowed X-ray absorption spectroscopy. As expected, microbial reduction processes developed in microbially-active systems with an initial pH of 10; however, during microbial incubations the pH dropped from 10 to ~ 7 , reflecting the high levels of microbial metabolism occurring in these systems. In microbially-active systems without added Fe(III), 90% sorption of Np(V) occurred within one hour with essentially complete removal by one day. In the ferrihydrite-amended systems, complete sorption of Np(V) to ferrihydrite occurred within one hour. For higher-activity sediments, X-ray absorption spectroscopy (XAS) at end points where Fe(II) ingrowth was observed confirmed that complete reductive precipitation of Np(V) to Np(IV) had occurred under similar conditions to low-level Np experiments. Finally, pre-reduced, Fe(III)-reducing sediments, with and without added Fe(III) and held at pH 10, were spiked with Np(V). These alkaline pre-reduced sediments showed significant removal of Np to sediments, and XAS confirmed partial reduction to Np(IV) with the no Fe system, and essentially complete reduction to Np(IV) in the Fe(III)-enriched systems. This suggested an indirect, Fe(II)-mediated pathway for Np(V) reduction under alkaline conditions. Microbial analyses using 16S rRNA gene pyrosequencing suggested a role for alkali-tolerant, Gram-positive Firmicutes in coupled Fe(III) reduction and Np immobilization in these experiments.

KEYWORDS: bioreduction, Np(V) reduction, intermediate-level waste, biomineralization, actinide biogeochemistry.

Introduction

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DOI: 10.1180/minmag.2015.079.6.04

NEPTUNIUM-237 is a long-lived (2.13×10^6 years) transuranic element with elevated radiotoxicity, which is present in intermediate level radioactive



The publication of this research has been funded by the European Union's European Atomic Energy Community's (Euratom) Seventh Framework programme FP7 (2007–2013) under grant agreements n°249396, SeclGD, and n°323260, SeclGD2.

wastes. As a long-lived decay product of ^{241}Am and ^{241}Pu , it is expected to become the dominant transuranic in radioactive wastes between 10^4 and 10^7 years (Lloyd and Renshaw, 2005; Kaszuba and Runde, 1999). In oxic environments neptunium exists predominantly as the neptunyl (Np(V)O_2^+) species which has the lowest affinity for surface sorption of all the transuranic elements (Lloyd and Renshaw, 2005; Kaszuba and Runde, 1999). Under reducing conditions, Np(IV) is expected to form and is prone to hydrolysis and sorption (Kaszuba and Runde, 1999). Microbial interactions with Np have been reported with immobilization by biosorption (Gorman-Lewis *et al.*, 2013; Songkasiri *et al.*, 2002), reductive immobilization to Np(IV) with the Fe(III) -reducing bacterium *Shewanella oneidensis* (Lloyd *et al.*, 2000), and in sediment microcosms under Mn(IV) - and Fe(III) -reducing conditions (Law *et al.*, 2010). Enzymatic Np(V) reduction was not observed in cultures of *Geobacter sulfurreducens*, suggesting that not all Fe(III) -reducing bacteria are capable of mediating direct (enzymatic) Np(V) reduction at circumneutral pH (Renshaw *et al.*, 2005).

As little is known about the biogeochemistry of Np(V) at elevated pH relevant to the disposal of cementitious intermediate-level radioactive wastes, this work was focused on the fate of Np(V) in microbially active, high-pH sediments collected from a lime working in Buxton, UK. These calcite-rich alkaline sediments are over 100 years old, and offer a robust model system to explore biogeochemical processes under the alkaline conditions that will predominate in many intermediate-level waste disposal situations (Williamson *et al.*, 2013; Rizoulis *et al.*, 2012; Bassil *et al.*, 2014). Biogeochemical transformations were studied in sediment microcosms that were spiked with 'tracer' concentrations of Np(V) (20 Bq ml^{-1} ; $3.3\ \mu\text{M}$), with and without added Fe(III) as ferrihydrite. The ferrihydrite additions were used to probe the impact of Fe biogeochemistry on radionuclide fate and behaviour as it will be an important component of wastes and also be present in the sub-surface facility as iron and steel drums and engineering components (Williamson *et al.*, 2013). The microcosms were sealed to allow anoxia to develop, and samples were periodically monitored for biogeochemical markers. Additionally, pre-reduced Fe(III) -reducing sediments, with and without added ferrihydrite (and held at pH 10), were incubated at 4°C to suppress microbial activity and reacted with Np(V) to explore abiotic/indirect reduction pathways. Selected experiments were also run at higher

Np(V) concentrations (2.5 kBq ml^{-1} ; $414\ \mu\text{M}$) to assess neptunium speciation using XAS techniques. Finally 16S rRNA gene pyrosequencing techniques were used to characterise the microbial communities involved in controlling the biogeochemical fate of Np(V) in these experiments.

Methods

Sample collection

Sediments were collected at the margins of a legacy lime working at Buxton, Derbyshire (Williamson *et al.*, 2013; Rizoulis *et al.*, 2012). Sediments and surface waters were transferred into sterile containers and stored at 4°C in darkness prior to use. The elemental composition of the sediment was determined by X-ray fluorescence (Axios Sequential X-ray Fluorescence Spectrometer).

Bioreduction microcosm construction

Microcosms were prepared in sterile serum bottles in triplicate with a sediment:solution ratio of 1:10. All systems were pH adjusted with HCl , then spiked with Np(V) (20 Bq ml^{-1} ($3.31\ \mu\text{M}$)). A groundwater control with Np(V) spiked into filtered ($<0.22\ \mu\text{m}$) site groundwater adjusted to pH 10 was also run. In low-level Np experiments, 5 g Buxton sediment and 50 ml surface water incubations with *no added Fe(III)* and *with added Fe(III)* were prepared. Sodium lactate (10 mM) and 1 g l^{-1} yeast extract were added to stimulate microbial activity, and Fe(III) was also added to a final concentration of 30 mmol l^{-1} as ferrihydrite in key experiments. The microcosms were flushed with argon, crimp sealed with butyl rubber stoppers, then progressive anoxia was allowed to develop at 20°C in the dark over one month. To explore further the mechanisms of radionuclide transformations in these systems under alkaline anoxic conditions, incubations with *no added Fe(III)* and *with added Fe(III)* were allowed to develop Fe(III) -reducing conditions for 28 days at 20°C and with pH control to 10. Microcosms were then moved to a refrigerator to minimize microbial activity for two days prior to spiking with Np(V) (20 Bq ml^{-1} ($3.31\ \mu\text{M}$)). The samples were then stored at 4°C for 14 days and the experiments were sampled periodically. A select set of higher-activity experiments was prepared for XAS analysis with 0.5 g sediment and 5 ml surface water, and spiked with 2.5 kBq ml^{-1} ($414\ \mu\text{M}$) Np(V) . These higher- Np activity experiments were run for oxic and pre-reduced sediments, both

with and without added ferrihydrite. The systems were then left to react for one hour in the case of oxic sediments, for two months during progressive reduction (pH \approx 8 at the end point), and for two weeks in the pre-reduced experiments (pH \approx 10 at the end point). At experimental end points, the pH and total Np in the supernatant were determined. During sediment incubations, the pH was not adjusted due to radiological safety considerations and the pH in the low Np microcosms dropped to between 7 and 8, and in XAS experiments to between 8 and 9. Throughout, sample manipulations were performed under anaerobic conditions as appropriate and using an aseptic technique.

Geochemical analyses: low level experiments

Sample slurries were analysed for pH and Eh using a calibrated meter and probes. To assess total non-refractory Fe, samples were digested in aqua regia followed by ICP-AES measurement on filtered supernatant (Perkin Elmer Optima 5300). Sediment biogenic Fe(II), and total bioavailable Fe were assessed by 0.5 N HCl extraction and quantification using the ferrozine colorimetric assay on a sub-sample of sediment slurry (Lovley and Phillips, 1987). Samples were then centrifuged (15,800 g) and the supernatant analysed for NO $_2^-$, NO $_3^-$, SO $_4^{2-}$ and organic acids (Dionex DX120) and for total Np (ICP-MS, Agilent 7500cx).

X-ray absorption spectroscopy (XAS) experiments

To identify the fate of Np(V), a set of higher-activity experiments were prepared for XAS analyses to give 500–1000 ppm on solids. Samples included oxic controls reacted for one hour (pH 10), end-point progressive anoxia experiments (pH 8), and end-point pre-reduced sediments (pH 10) at 4°C, all with and without added ferrihydrite. After reaction, sediment pellets were transferred to a triple container sample cell and stored under argon at –80°C prior to analysis. Measurements were conducted at the ANKA INE Beamline where neptunium L_{III} -edge spectra were collected in fluorescence mode using a 5 pixel solid-state detector and Ge(422) monochromator at 77 K using a cryostat (Law *et al.*, 2010). Energy calibration was completed by measurement of a Y foil. XANES spectra were collected for all samples, and where possible, EXAFS data were also collected: a total of 7–10 spectra were collected

per sample to allow averaging to improve the signal to noise ratio. Background subtraction, data normalization and fitting of the EXAFS spectra were performed using *ATHENA* and *ARTEMIS* (Ravel and Newville, 2005). Linear combination fitting of XANES data was performed to further indicate Np oxidation state using Np(V) and Np(IV) standards (Rossberg *et al.*, 2014; Hennig, 2007) in *ATHENA* (Ravel and Newville, 2005). The EXAFS data could be fitted in k^3 space between 3 and 8 Å $^{-1}$ and shells were only included in the model fit if the goodness of fit (R) was improved by >5% and the data were statistically significant as assessed by the reduced χ^2 test.

16S rRNA gene amplicon pyrosequencing and data analysis

Bacterial community structure was examined in the oxic Buxton sediment and also in the *no added Fe(III)*, and *with added Fe(III)* microcosms at low- and high-Np concentrations at incubation end points. DNA was isolated from 0.2 g of sediment using the MoBio PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). PCR for amplicon pyrosequencing was performed using tagged fusion bacterial primers 27F and 338R (Lane, 1991; Daims *et al.*, 1999), targeting the V1-V2 hypervariable region of the bacterial 16S rRNA gene. The fusion forward primer (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGN-NNNNNNNNNAGAGTTTGATGTTGGCTCAG-3') contained the 454 Life Sciences "Lib-L Primer A", a 4 base "key" sequence (TCAG), a unique ten-base pair multiplex identifier (MID) sequence for each sample, and bacterial primer 27F. Roche's MID-14 barcode (CGAGAGATAC) was used for the *low Np no added Fe(III)*, MID-16 (TCACGTACTA) for the *low Np with added Fe(III)*, MID-17 (CGTCTAGTAC) *high Np no added Fe(III)*, MID-18 (TCTACGTAGC) *high Np with added Fe(III)*, and MID-13 (CATAGTAGTG) for the oxic Buxton sediment. The reverse fusion primer (5'-CC TATCCCCTGTGTGCCTTGGCAGTCTCAGTGC TGCCTCCCGTAGGAGT-3') contained the 454 Life Sciences "Lib-L Primer B", a 4 base "key" sequence (TCAG), and bacterial primer 338R. The pyrosequencing run was performed at The University of Manchester sequencing facility using a Roche 454 Life Sciences GS Junior system. Details of the PCR procedure and pyrosequencing analysis have been previously outlined (Williamson *et al.*, 2014). The pyrosequencing reads from this study

will be deposited in the NCBI Sequence Read Archive (SRA).

Results and discussion

Sediment and surface water characteristics

The sediment consisted of calcite, with minor quartz and ankerite (Williamson *et al.*, 2013, 2014). The elemental composition was dominated by Ca (52.8 wt.%), with significant concentrations of Si (1.1 wt.%), Al (0.51 wt.%) and Fe (0.15 wt.%) with only trace

Mn (<0.007%) detected in sediments. The Fe measured in the aqua regia digest of the sample was determined at 0.51 g kg^{-1} (9.1 mM kg^{-1}) and the bioavailable Fe was estimated at 0.27 g kg^{-1} (4.8 mM kg^{-1}).

Biogeochemistry in sediment microcosms

To explore the biogeochemistry of neptunium under anoxic, initial pH 10 conditions, microcosms were constructed using calcite dominated sediments and surface waters from a high pH lime

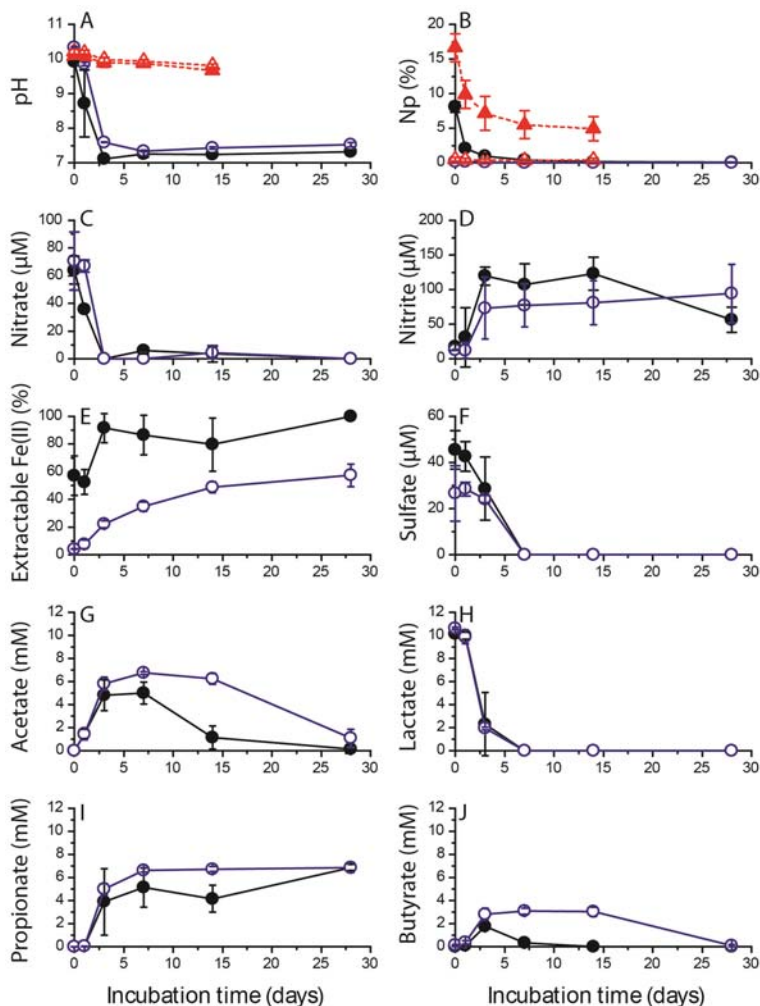


FIG. 1. Bioreduction of Buxton sediments with *no added Fe(III)* (●) and *with added Fe(III)* (○) showing (A) pH; (B) % Np in solution; (C) NO_3^- ; (D) NO_2^- (E) percentage of 0.5 N HCl extractable Fe present as Fe(II) (%), (F) SO_4^{2-} , (G) acetate, (H) lactate, (I) propionate and (J) butyrate. Dashed lines in A and B represent pre-reduced controls with *no added Fe(III)* (▲) and *with added Fe(III)* (△). Error bars are 1σ of triplicate results (where not shown, errors are within the symbol size).

workings site in the UK (Williamson *et al.*, 2013). The sediments have been well characterized and have been used in recent work focusing on the biogeochemistry of Fe(III) and U(VI), where the pH was maintained at pH 10 (Williamson *et al.*, 2013, 2014). The experiments here were not pH controlled due to issues with radiological safety, and this provides insight into an evolving waste environment for ILW where microbial degradation of organics will occur (e.g. Bassil *et al.*, 2014) and microbially-driven acidification reactions may reduce the pH with time. In all progressive anoxia experiments, a range of anaerobic biogeochemical processes developed including microbial Fe(III) reduction (Fig. 1). Throughout incubations Mn, which is implicated in Np biogeochemistry (Law *et al.*, 2010), was undetectable in solution presumably reflecting its trace concentration in the sediments. The pH of the microcosms dropped to between 7 and 8 and the concentrations of organic acids increased in porewaters in the progressive anoxia *no added Fe(III)* and *with added Fe(III)* experiments (Fig. 1). The reduced pH was clearly linked to microbial metabolism and is likely to be due to both production of volatile fatty acids and generation of CO₂. For the *no added Fe(III)* experiment, there was complete nitrate removal by day 1 and complete Fe(III) reduction by day 3, and a fall in pH to 7.1 and Eh from +179±1 mV at the start of the experiment, to -82.0±12 mV by day 14 (Fig. 1). Interestingly, sulfate removal was also observed by day 7, suggesting sulfate reduction was occurring in these systems (Fig.1). This is in contrast to systems maintained at pH 10 (Williamson *et al.*, 2014), where sulfate reduction is energetically unfavourable (Rizoulis *et al.*, 2012), and highlights the potential for localized acidification due to biogeochemical processes in radioactive waste disposal. In the systems *with added Fe(III)*, complete nitrate removal occurred by day 3 and nitrite concentrations then increased (Fig. 1). The pH dropped to 7.6 by day 3, and 0.5 N HCl extractable Fe(II) concentrations reached 8.84 ± 0.54 mM (~50% of total bioavailable Fe(III)) by day 14. In addition, essentially complete removal of sulfate had occurred by day 7, again confirming that sulfate-reducing conditions were generated rapidly in these systems (Fig. 1).

Neptunium fate during bioreduction

In the no added iron experiments, Np(V) (20 Bq ml⁻¹; 3.3 µM) was added to filter sterilized

(<0.2 µm) oxic surface water samples, microbially-active sediment slurry microcosms as well as pre-reduced Fe(III)-reducing sediments held at 4°C to minimize microbial activity. The solubility of Np(V) in filtered sterilized Buxton surface waters was high, with 84 ± 11% of the spike remaining in solution after two days. In oxic sediments, significant Np(V) sorption occurred after one hour (~95% removed) and the Np concentration was then constant over one week, suggesting significant abiotic uptake of Np(V) to these sediments at pH 10. In progressive anoxia microcosms with *no added Fe(III)*, again, significant Np sorption occurred after one hour (~90% was removed), and by day 14 where robust Fe(III) and sulfate reduction was evident, complete removal of Np was observed (Fig. 1). In *no added Fe(III)* systems, again significant Np(V) removal occurred within one hour (~83%) and removal continued over two weeks to a level of ~94% removal in pre-reduced, Fe(III)-reducing sediments incubated at 4°C, but removal was not as effective as in microbially active

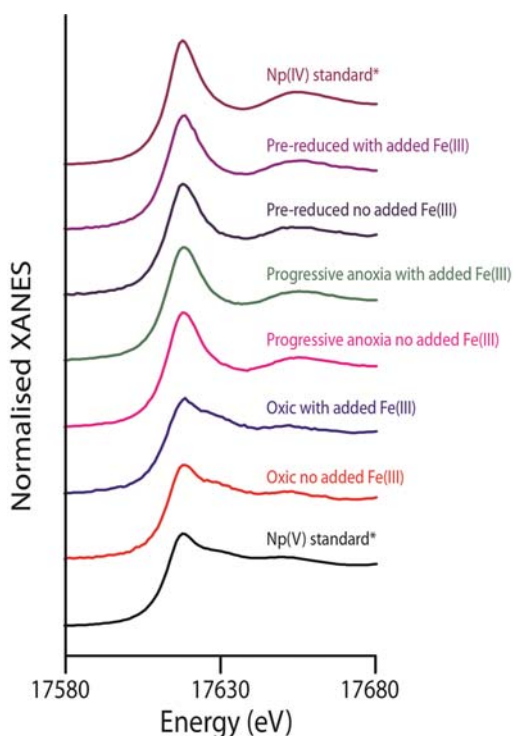


FIG. 2. ²³⁷Np L_{III}-edge XANES stack plot of oxic, progressive anoxia, and pre-reduced experiments with Np(V)O₂⁺ and Np(IV)O₂ reference spectra.

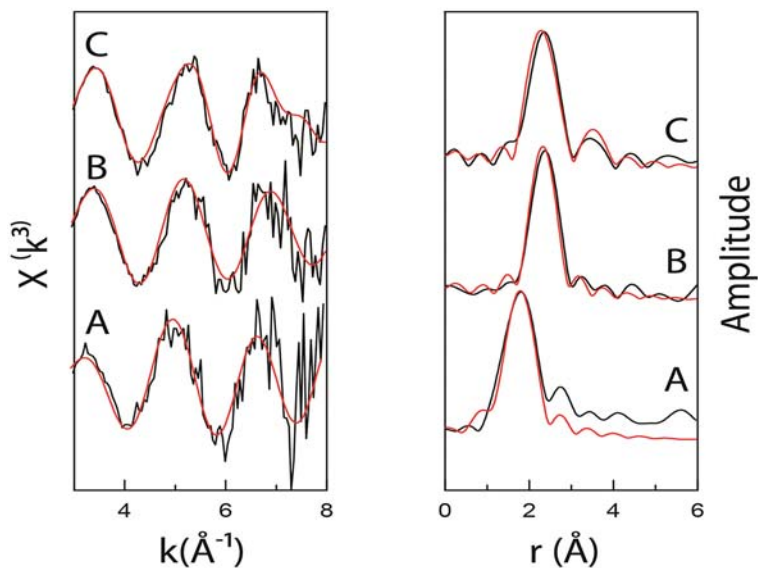


FIG. 3. k^3 weighted ^{237}Np L_{III} -edge EXAFS spectra (left) and Fourier transform (right) for: (A) oxic sediments; (B) progressive anoxia *no added Fe(III)*; and (C) progressive anoxia *with added Fe(III)*. The best fit model (red line) overlays the experimental data (black lines). The fit parameters are given in Table 1.

experiments (Fig. 1). We are confident that microbial activity in these experiments was significantly reduced as the pH remained between 9.5 and 10 over the two-week incubation, and in contrast to the systems held at room temperature (Fig. 1*a*). Np L_{III} -edge XANES and, where possible, EXAFS spectra were collected on parallel experiments at higher (2.5 kBq ml^{-1} , $414 \mu\text{M}$) concentrations and after two months of incubation, these systems mirrored the end point of the lower activity experiment Np behaviour. The oxic sediment showed a Np(V) like XANES spectrum, suggesting sorption of Np(V) . By contrast, the progressive anoxia *no added Fe(III)* end-point sample showed a Np(IV) -like XANES spectrum confirming reductive scavenging of Np(IV) to solids (Fig. 2). Linear combination fitting of the endmember XANES spectra from the *no added Fe(III)* progressive anoxia sample was performed, against Np(V) and Np(IV) standards, and Np(IV) was dominant in the spectrum with an 84% contribution from the model Np(IV) spectrum. The Np EXAFS data were fitted between k^3 3–8 \AA^{-1} , and were best fitted with one shell of oxygen backscatterers of 80 at 2.34 \AA indicative of Np(IV) (Combes *et al.*, 1992) and with no evidence for a short dioxygenyl interaction (for Np(V) 2O at 1.91 \AA ; Clark *et al.*, 1996) confirming the dominance of Np(IV) (Fig. 3; Table 1). When

Np was added to pre-reduced sediments at pH 10 with *no added Fe(III)* incubated at 4°C , the XANES data suggested Np(IV) was dominant with linear combination fitting suggesting $\sim 75\%$ Np(IV) (Fig. 2). The increased retention of Np in the progressive anoxia experiments compared to the pre-reduced experiments may be due to differences in the geochemical conditions: experiments were at pH 8 in the progressive systems and 8.9 in the pre-reduced experiments. Overall, these results suggest a significant pathway for abiotic reduction of Np(V) presumably via interactions with biogenic Mn(II) or Fe(II) (Law *et al.*, 2010), and differences in reactivity of the pre-reduced versus progressive anoxia systems in the context of biotic and abiotic/indirect processes warrant further investigation.

In the with added iron systems, oxic, progressive anoxia and pre-reduced sediments showed complete Np(V) sorption by one hour suggesting that Np(V) sorption was affected significantly by the addition of ferrihydrite, as expected from previous work where Np is highly reactive towards these phases (Girvin *et al.*, 1991; Fig. 1). For the oxic sample, the EXAFS data were fitted with two axial oxygen backscatterers at 1.91 \AA , consistent with the Np(V) dioxygenyl species (Clark *et al.*, 1996). Again, the XANES data from these experiments

TABLE 1. EXAFS fitting results for oxic with added Fe(III), progressive anoxia no added iron, and progressive anoxia with added iron. CN denotes the coordination number, r is the interatomic distance (Å) and σ^2 is the Debye Waller factor (Å²). ΔE_0 represents the shift in energy from the calculated Fermi level. S_0^2 denotes the amplitude factor (constrained between 0.85 and 1.05 in reduced samples and set to 1 in the oxic sample). R is the goodness of the fit and χ^2 is the reduced χ^2 value. Numbers in parentheses are the standard deviation on the last decimal place.

Sample	Shell	Path	CN	r (Å)	$2\sigma^2$ (Å ²)	ΔE_0 (eV)	S_0^2	R	χ^2																									
Oxic no added Fe(III)	1	O _{ax}	2	1.91 (4)	0.036 (2)	8.69 ± 3.62	1	0.024	8.4																									
	2	O _{eq}	6	2.39 (3)	0.008 (4)					Reduced, no added Fe(III)	1	O _{eq}	8	2.33 (2)	0.015 (6)	5.71 ± 1.78	0.92 ± 0.16	0.029	20	2	Fe	2	3.44 (5)	0.030 (7)	Reduced with added Fe(III)	1	O _{eq}	8	2.34 (2)	0.022 (4)	6.94 ± 1.88	1.05 ± 0.23	0.025	35
Reduced, no added Fe(III)	1	O _{eq}	8	2.33 (2)	0.015 (6)	5.71 ± 1.78	0.92 ± 0.16	0.029	20																									
	2	Fe	2	3.44 (5)	0.030 (7)																													
Reduced with added Fe(III)	1	O _{eq}	8	2.34 (2)	0.022 (4)	6.94 ± 1.88	1.05 ± 0.23	0.025	35																									
	2	Fe	2	3.44 (5)	0.030 (7)																													

showed a clear progression from Np(V) in oxic sediments to Np(IV) in progressive anoxia and pre-reduced Fe(III)-reducing sediments (Fig. 2), with linear combination fitting confirming Np(IV) was dominant. EXAFS analyses supported these observations, with 8 oxygen backscatterers at 2.41 Å typical of Np(IV), and two Fe backscatterers at 3.44 Å providing the best fit which satisfied the reduced χ^2 test (Fig. 3; Table 1). This suggested a Np-Fe interaction similar to that observed in Fe(III)-reducing sediments at circumneutral pH (Law *et al.*, 2010).

16S rRNA gene amplicon pyrosequencing

Pyrosequencing of the unamended Buxton sediment revealed a diverse community with 2709 reads (after de-noising and removal of short chimeric reads) grouped to 187 OTUs (at 97% sequence ID similarity) affiliated to 13 bacterial phyla. These were dominated by β -Proteobacteria (19.7%), Bacteroidia (14.5%), γ -Proteobacteria (11.8%) and Firmicutes (16.6%) (Fig. 4). After 28 days incubation the *no added Fe(III)* sediment community generated 7054 reads and 204 OTUs. Sequence analyses showed that there was a clear enrichment in Bacteroidia (62.4%) and Clostridia (16.81%) (Fig. 4), with a close match to an Fe enrichment clone from the Scheldt estuary (12.2% abundance, 99% sequence match) (Lin *et al.*, 2007). In the parallel high-level Np experiments, a more diverse community was observed, dominated by β -Proteobacteria (27%) and Clostridia (25%) (Fig. 4) with a 100% sequence match to *Acidovorax* sp. p4, reported in unpublished work from a mine drainage site in China (Lu *et al.*, unpublished). The difference in community shifts here may be reflective of the higher pH recorded in these high-Np systems (pH 8), or increased radiotoxicity of the Np when used at higher concentrations (Reed *et al.*, 2010). A very different community shift was observed however in the *with added Fe(III)* system after 28 days incubation, with 6797 reads and 136 OTUs, with Clostridia (57.3%) dominating (Fig. 4). A similar community shift was observed in the parallel experiments with high Np (71.1% Clostridia), with a close match to *Clostridium* sp. PS-6, isolated from a nuclear power plant (Garcia *et al.*, unpublished). These community shifts may indicate that Fe(III) reduction is driven by alkali-tolerant Gram-positive Firmicutes similar to experiments maintained at pH 10 (Williamson *et al.*, 2014).

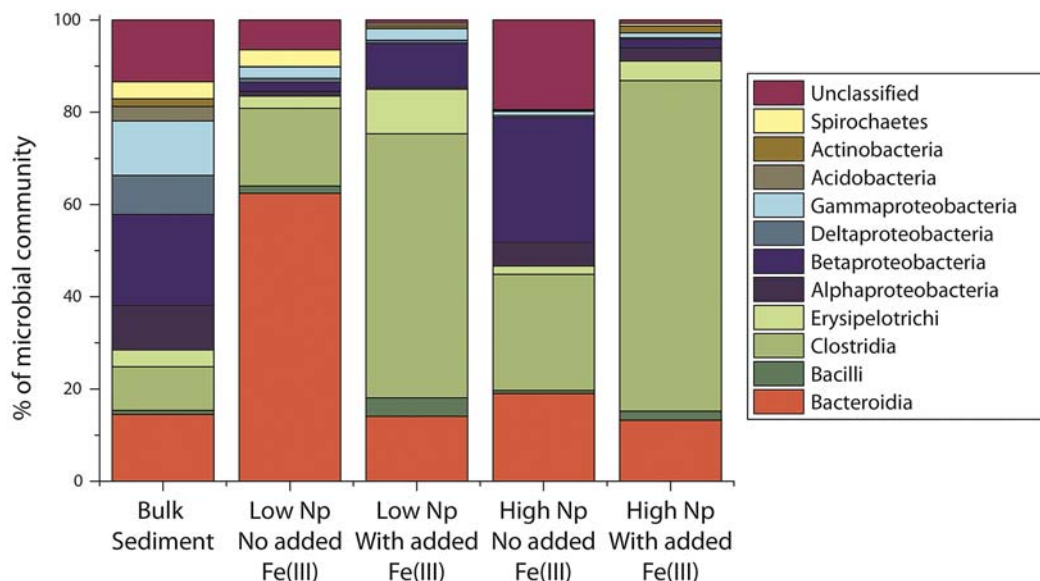


FIG. 4. Bacterial phylogenetic diversity at the Phylum level for the oxic sediment and end-point experiments for low Np *no added Fe(III)* and *with added Fe(III)* and high Np *no added Fe(III)* and *with added Fe(III)*. Only the phyla with more than 1% of the total number of reads are shown. Abundance at the class level is shown for Firmicutes (green hues) and Proteobacteria (blue hues).

Conclusions

On reaction with oxic sediments at pH 10 in the absence of added ferrihydrite, significant sorption of Np(V) to sediments was observed and XAS confirmed Np(V) on solids. In the presence of added ferrihydrite, oxic sediments showed a high reactivity for Np(V) and again XAS confirmed Np(V) on solids in oxic sediments. In the no added Fe(III) progressive anoxia system, development of bioreduction caused a pH fall to between 7 and 8 and essentially complete removal of Np from solution occurred by 7 days. In the pre-reduced control at pH 8.9 there was significant, but not complete Np sorption (94% over two weeks). XAS analysis confirmed that reduction to Np(IV) on solids occurred in both progressive and pre-reduced no added Fe(III) systems. In the systems with added Fe(III), essentially complete Np(V) sorption was observed within one hour in both progressive and pre-reduced experiments, and XAS analyses on end-point samples confirmed reduction to Np(IV) over the time course of the experiment. Overall, this suggests a controlling biogeochemical influence on Np mobility in these systems. Additionally, the impact of microbial metabolism on pH control in these experiments is noteworthy, with organic acid

production and CO₂ generation the most likely causes for the significant decrease in pH over the first week of incubation. This is relevant to ILW disposal, as cellulose is a significant component of the wastes and recent work has highlighted that microbially mediated degradation of organics is possible at elevated pH (Bassil *et al.*, 2014, 2015), and this may in turn be coupled to anaerobic processes including Fe(III) reduction.

Acknowledgements

This work was funded as part of the NERC BIGRAD consortium, NERC grant NE/H007768/1. JRL acknowledges financial support from the Royal Society. The INE beamline at ANKA synchrotron light source, Germany, was accessed via Actinet and EnvRadNet. The authors thank Dr Jörg Rothe for assistance at ANKA and Alistair Bewsher and Paul Lythgoe for analytical geochemistry support.

References

- Bassil, N.M., Bryan, N. and Lloyd, J.R. (2014) Microbial degradation of isosaccharinic acid at high pH. *The ISME Journal*, 1–11.

- Bassil, N.M., Bewsher, A.D., Thompson, O.R. and Lloyd, J.R. (2015) Microbial degradation of cellulosic material under ILW-simulated conditions. *Mineralogical Magazine*, **79**, DOI: 10.1180/minmag.2015.079.6.18.
- Clark, D.L., Conradson, S.D., Ekberg, S.A., Hess, N.J., Neu, M.P., Palmer, P.D., Runde, W. and Tait, C.D. (1996) EXAFS studies of pentavalent neptunium carbonate complexes. Structural elucidation of the principal constituents of neptunium in groundwater environments. *Journal of the American Chemical Society*, **118**, 2089–2090.
- Combes, J.-M., Chisholme-Brause, Catherine, J., Brown Jr, G.E., Parks, G.A., Conradson, S.D., Eller, P.G., Trlay, I.R., Hobart, D.E. and Meljer, A. (1992) EXAFS spectroscopic study of neptunium(V) sorption at the α -FeOOH water interface. *Environmental Science & Technology*, **26**, 376–382.
- Daims, H., Brühl, A., Amann, R., Schleifer, K.H. and Wagner, M. (1999) The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology*, **22**, 434–44.
- Girvin, D.C., Ames, L.L., Schwab, A.P. and McGarrath, J.E. (1991) Neptunium adsorption on synthetic amorphous iron oxyhydroxide. *Journal of Colloid and Interface Science*, **141**, 67–78.
- Gorman-Lewis, D., Jensen, M.P., Harold, Z.R. and Hertel, M.R. (2013) Complexation of neptunium(V) with *Bacillus subtilis* endospore surfaces and their exudates. *Chemical Geology*, **341**, 75–83.
- Hennig, C. (2007) Evidence for double-electron excitations in the L₃-edge X-ray absorption spectra of actinides. *Physical Review B*, **75**, 1–7.
- Kaszuba, J.P. and Runde, W.H. (1999) The aqueous geochemistry of neptunium: Dynamic control of soluble concentrations with applications to nuclear waste disposal. *Environmental Science & Technology*, **33**, 4427–4433.
- Lane, D.J. (1991) 16S/23S rRNA sequencing. Pp. 115–175 in: *Nucleic Acid Techniques in Bacterial Systematics* (E. Stackebrandt and M. Goodfellow, editors). New York: John Wiley and Sons.
- Law, G.T.W., Geissler, A., Lloyd, J.R., Livens, F.R., Boothman, C., Begg, J.D.C., Denecke, M.A., Rothe, J., Dardenne, K., Burke, I.T., Charnock, J.M. and Morris, K. (2010) Geomicrobiological redox cycling of the transuranic element neptunium. *Environmental Science & Technology*, **44**, 8924–8929.
- Lin, B., Hyacinthe, C., Bonneville, S., Braster, M., Van Cappellen, P. and Röling, W.F.M. (2007) Phylogenetic and physiological diversity of dissimilatory ferric iron reducers in sediments of the polluted Scheldt estuary, northwest Europe. *Environmental Microbiology*, **9**, 1956–68.
- Lloyd, J. and Renshaw, J. (2005) Microbial transformations of radionuclides: fundamental mechanisms and biogeochemical implications. Pp. 205–240 in: *Metal Ions in Biological Systems* (A. Siegal and R.K.O. Siegal, editors). New York: M. Dekker.
- Lloyd, J.R., Yong, P. and Macaskie, L.E. (2000) Biological reduction and removal of Np(V) by two microorganisms. *Environmental Science & Technology*, **34**, 1297–1301.
- Lovley, D.R. and Phillips, E.J. (1987) Rapid assay for microbially reducible ferric iron in aquatic sediments. *Applied and Environmental Microbiology*, **53**, 1536–1540.
- Ravel, B. and Newville, M. (2005) ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *Journal of Synchrotron Radiation*, **12**, 537–541.
- Reed, D.T., Deo, R.P. and Rittmann, B.E. (2010) Subsurface interactions of actinide species with microorganisms. Pp. 3595–3663 in: *Chemistry of the Actinides*, 4th edition (L.R. Morse, N.M. Edelstein and J. Fuger, editors) Netherlands, Springer.
- Renshaw, J.C., Butchins, L.J.C., Livens, F.R., May, I., Charnock, J.M. and Lloyd, J.R. (2005) Bioreduction of uranium: environmental implications of a pentavalent intermediate. *Environmental Science & Technology*, **39**, 5657–5660.
- Rizoulis, A., Steele, H.M., Morris, K. and Lloyd, J.R. (2012) The potential impact of anaerobic microbial metabolism during the geological disposal of intermediate-level waste. *Mineralogical Magazine*, **76**, 3261–3270.
- Rosberg, A., Scheinost, A.C., Schmeisser, N., Rothe, J., Kaden, P., Schild, D., Wiss, T. and Daehn, R. (2014) AcReDaS, an Actinide Reference Database for XAS, EELS, IR, Raman and NMR Spectroscopy. Available at: <https://www.hzdr.de/acredas>.
- Songkasiri, W., Reed, D.T. and Rittmann, B.E. (2002) Bio-sorption of neptunium (V) by *Pseudomonas fluorescens*. *Radiochimica Acta*, **90**, 785–789.
- Williamson, A.J., Morris, K., Shaw, S., Byrne, J.M., Boothman, C. and Lloyd, J.R. (2013) Microbial reduction of Fe(III) under alkaline conditions relevant to geological disposal. *Applied and Environmental Microbiology*, **79**, 3320–3326.
- Williamson, A.J., Morris, K., Charnock, J.M., Law, G.T.W., Rizoulis, A. and Lloyd, J.R. (2014) Microbial reduction of U(VI) under alkaline conditions; implications for radioactive waste disposal. *Environmental Science & Technology*, **48**, 13549–13556.