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Neptunium and manganese biocycling in nuclear legacy sediment systems

Clare L. Thorpe [a], Katherine Morris [a], Jonathan R. Lloyd [a], Melissa A. Denecke [b], Kathleen A. Law [b], Kathy Dardenne [c], Christopher Boothman [a], Pieter Bots [a], Gareth T.W. Law [b, *]

[a] Research Centre for Radwaste Disposal and Williamson Research Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Manchester M13 9PL, UK
[b] Centre for Radiochemistry Research, School of Chemistry, The University of Manchester, M13 9PL, UK
[c] Karlsruhe Institute of Technology, Institut Fur Nukleare Entsorgung, D-76021 Karlsruhe, Germany

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ABSTRACT

Understanding the behaviour of the highly radiotoxic, long half-life radionuclide neptunium in the environment is important for the management of radioactively contaminated land and the safe disposal of radioactive wastes. Recent studies have identified that microbial reduction can reduce the mobility of neptunium *via* reduction of soluble Np(V) to poorly soluble Np(IV), with coupling to both Mn- and Fe(III)-reduction implicated in neptunyl reduction. To further explore these processes Mn(IV) as δMnO_2 was added to sediment microcosms to create a sediment microcosm experiment "poised" under Mn-reducing conditions. Enhanced removal of Np(V) from solution occurred during Mn-reduction, and parallel X-ray absorption spectroscopy (XAS) studies confirmed Np(V) reduction to Np(IV) commensurate with microbially-mediated Mn-reduction. Molecular ecology analysis of the XAS systems, which contained up to 0.2 mM Np showed no significant impact of elevated Np concentrations on the microbial population. These results demonstrate the importance of Mn cycling on Np biogeochemistry, and clearly highlight new pathways to reductive immobilisation for this highly radiotoxic actinide.

1. Introduction

Internationally, deep geological disposal is being considered as the long-term management and disposal option for higher activity radioactive wastes (HAW). A fundamental knowledge of reactions between radionuclides and geomedia is essential to underpin the safety case for geodisposal. Neptunium is a key risk-driving radionuclide in HAW due to its long half-life (^{237}Np $t_{½} = 2.1 \times 10^6$ years), ingrowth from ^{241}Am, high radiotoxicity, and relatively high solubility as Np(V). Indeed, Np is potentially the most mobile transuranic species in environments pertinant to deep geological disposal (e.g. Choppin and Stout, 1989; Kaszuba and Runde, 1999; Lloyd et al., 2000; Choppin, 2007; Law et al., 2010); further, Np is a persistant contaminant at or near nuclear sites (e.g. Cantrell, 2009; Morris et al., 2000; Stamper et al., 2013).

Neptunium is redox active and it's environmental mobility can be affected by the biogeochmistry and redox conditions in the subsurface (Kaszuba and Runde, 1999; Lloyd et al., 2002; Choppin, 2007; Law et al., 2010). Under oxidising conditions Np is stable in solution as the soluble neptunyl cation, NpO_2^+, whilst under anaerobic conditions Np can be reduced to poorly soluble Np(IV) species (Kaszuba and Runde, 1999; Moyes et al., 2002; Llorens et al., 2005; Law et al., 2010; Bach et al., 2014). In the subsurface, microbial respiration can induce anaerobic conditions under which metals and radionuclides can be reduced (Lloyd and Renshaw, 2005). The development of bioreducing conditions is increasingly recognised as likely to be significant in the deep subsurface around a geological disposal facility (Pedersen, 2000; Fredrickson and Balkwill, 2006; Rizoulis et al., 2012; Williamson et al., 2013; Behrends et al., 2012), and is the basis for remediation of contaminated land where problematic radionuclides (e.g. Tc and U) may be reduced either enzymatically or indirectly *via* interactions with reduced species (e.g. Fe(II): Lloyd et al., 2002; Lloyd, 2003; Gadd, 2010; Newsome et al., 2014).

* Corresponding author.
E-mail address: gareth.law@manchester.ac.uk (G.T.W. Law).

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The ability of microorganisms to enzymatically reduce Np(V) to Np(IV) has been demonstrated in pure culture experiments (Lloyd et al., 2000; Icopini et al., 2007) although some microorganisms are unable to facilitate enzymatic Np(V) reduction (Songkasiri et al., 2002; Renshaw et al., 2005). In contrast, Gorman-Lewis et al. (2005) inferred potential non-enzymatic reduction of Np(V) to explain trends in sorption/desorption experiments conducted with *Bacillus subtilis*. Toxicity effects on selected metal-reducing bacteria are also of interest as studies with indigenous microorganisms highlight the tolerence of microorganisms to mM concentrations of Np (Law et al., 2010; Ams et al., 2013), whilst in pure culture experiments no toxicity effects were observed at Np concentrations less than 2 mM (Ruggiero et al., 2005). In sediment systems, reductive immobilisation of Np(V) to Np(IV) has been observed during development of sediment anoxia with microbial metal reduction implicated in the reaction and with indirect (abiotic) reduction by Fe(II) shown to be possible (Law et al., 2010).

Manganese is ubiquitous in soils and rock forming minerals and therefore, although Np interactions with Mn minerals have been studied previously (Wilk et al., 2005), a deeper understanding of Np(V) behaviour during early metal reduction (Mn- and Fe(III)-reduction) is essential in understanding its environmental behaviour in both deep and shallow subsurface environments. In addition, the potential importance of Mn in environmental actinide chemistry is increasingly recognised with Mn linked to both Pu and U cycling (Powell et al., 2006; Hu et al., 2010; Wang et al., 2013, 2014). Here we examine the behaviour of Np in sediment systems amended with labile Mn(IV) (δMnO_2) to allow microcosms to develop a period of extended or "poised" Mn reduction (Lovley and Phillips, 1988). As well conducting experiments at low Np concentrations, we also collected XAS data from parallel experiments run at higher concentrations of Np. This allowed assessment of Np speciation and local-coordination under defined biogeochemical conditions. Finally, 16S rRNA gene analysis was performed to assess the response of the indigenous microbial communities to elevated Np concentrations.

2. Experimental section

2.1. Safety

Neptunium (^{237}Np) is a high radiotoxicity alpha-emitting radionuclide with beta/gamma emitting progeny. Work can only be conducted by trained personnel in a certified, properly equipped radiochemistry laboratory, following appropriate risk assessment. The possession and use of radioactive materials is subject to statutory control.

2.2. Sample collection

Sediments were collected from an area located ~2 km from the Sellafield reprocessing site in Calder River Valley, Cumbria (Lat 54°26′30 N, Long 03°28′09 W). Sediments were representative of the Quaternary unconsolidated alluvial flood-plain deposits that underlie the Sellafield site (Law et al., 2010) and were collected in sterile containers, sealed, and stored at 4 °C prior to use (<1 month).

2.3. Bioreduction microcosms with low NpO_2^+ concentrations

Sediment microcosms (10 ± 0.1 g Sellafield sediment, 100 ± 1 ml groundwater; in triplicate) were prepared using a synthetic groundwater representative of the Sellafield region (Wilkins et al., 2007) but with added nitrate and manganese (2 mM $NaNO_3$, 2 mM δMnO_2) and with a total 0.17 mmols of bioavailable Fe(III) in the sediment. Sodium acetate was also added in stoichiometric excess (10 mM) as an electron donor, the groundwater was sterilised (autoclaved for 1 h at 120 °C), purged with filtered 80%/20% N_2/CO_2, and pH adjusted to pH 7 (*via* drop-wise addition of 0.5 M HCl or 1 M NaOH). Sediments and sterile groundwater were then added to sterile 120 ml glass serum bottles (Wheaton Scientific, USA) and sealed with butyl rubber stoppers using aseptic technique. Neptunium, as $^{237}NpO_2^+$ (20 Bq ml^{-1}; 3.2 μM; oxidation state verified by UV–Vis analysis) was then spiked into each microcosm; thereafter, the microcosms were incubated anaerobically at 21 °C in the dark for 38 days. Throughout the incubation, sediment slurry was periodically extracted using aseptic technique, under an O_2-free, Ar atmosphere. The sediment slurry was centrifuged (15,000 g; 10 min) to separate sediment and porewater samples and ~0.5 g of sediment was stored at −80 °C for later microbiological characterisation.

2.4. Geochemical analyses

During microcosm sampling, total dissolved Mn and Fe concentrations were measured with standard UV–Vis spectroscopy methods on a Jenway 6715 spectrophotometer (Lovley and Phillips, 1987; Goto et al., 1997; Viollier et al., 2000). Aqueous NO_3^-, SO_4^{2-}, ammonium and acetate were measured by ion chromatography (Dionex ICS5000). Total bioavailable Fe(III) and the proportion of extractable Fe(II) in the sediment was estimated by digestion of 0.1 g of sediment in 5 ml of 0.5 N HCl for 60 min followed by the ferrozine assay, with and without added hydroxylammonium chloride (Lovley and Phillips, 1987; Viollier et al., 2000). The pH and Eh were measured with an Orion 420A digital meter and calibrated electrodes. Standards were routinely used to check the reliability of all methods and typically, calibration regressions had $R^2 \geq 0.99$. The elemental composition and bulk mineralogy of the sediment were determined by XRF (Thermo ARL 9400) and XRD (Philips PW 1050). Total organic carbon and total inorganic carbon were determined on a LECO CR-412 Carbon Analyser. The total ^{237}Np concentration in solution was measured by ICP-MS (Agilent 7500cx) using ^{232}Th as the internal standard.

2.5. XAS experiments

Experiments were prepared to allow direct determination of Np speciation and local coordination environment in sediments under different geochemical conditions using X-ray Absorption Spectroscopy (XAS). Here, the elevated concentration of Np required for direct spectroscopic characterisation (0.2 mM Np(V) as NpO_2^+ in 0.07 M HCl) was added to microcosms containing 1 g of sediment and 10 ml of groundwater that were poised at oxic, nitrate-, Mn-, Fe(III)-, and sulphate-reducing conditions, respectively. After Np(V) addition, the microcosms were left to incubate for 1 week in the dark at 7 °C prior to geochemical sampling and subsequent freezing at −80 °C. Two additional Mn-reducing XAS systems were also established where sediments had 2 mM of δMnO_2 added: (i) 0.2 mM NpO_2^+ was added to an oxic microcosm that was then left to progress to Mn-reducing conditions (verified by the presence of Mn in porewaters and the absence of detectable 0.5 N extractable Fe(II) in sediments) before freezing at −80 °C, and (ii) a parallel Mn-reducing microcosm (again with no detectable 0.5 N extractable Fe(II) in sediments) that was sterilised by autoclaving (1 h at 120 °C) prior to the addition of 0.2 mM NpO_2^+, and which was frozen at −80 °C 2 days after Np(V) addition. For XAS analysis, sediment samples were defrosted, centrifuged, and ~0.5 g of sediment was packed (under anaerobic atmosphere if necessary) into airtight sample containers which were then triple contained and frozen until analysis. XAS analysis was conducted at the INE Beamline for