

# The potential impact of anaerobic microbial metabolism during the geological disposal of intermediate-level waste

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## ABSTRACT

Microbial metabolism has the potential to control the biogeochemistry of redox-active radionuclides in a range of geodisposal scenarios. In this study, sediments from a high pH lime workings site were incubated under carefully controlled anaerobic conditions, at a range of alkali pH values with added electron donors and electron acceptors, to explore the limits and rates of bioreduction in a sediment system analogous to intermediate-level nuclear waste. There was a clear succession in the utilization of electron acceptors (in the order nitrate > Fe(III)-citrate > Fe(III) oxyhydroxide > sulfate), in accordance with calculated free energy yields and Eh values over the pH range 10–12. The rate and extent of bioreduction decreased at higher pH, with an upper limit for the processes studied at pH 12. The biochemical limits for such processes are discussed, alongside the potential impact of such forms of microbial metabolism on the solubility of a range of redox active radionuclides that will feature heavily in the safety case for the geological disposal of intermediate-level nuclear waste.

**KEYWORDS:** safety case, alkaliphile, geodisposal, actinide, fission product, ILW.

## Introduction

THE UK has an extensive legacy of radioactive waste from more than 60 years of military and civil nuclear technology, and this inventory will increase with future activities, including the potential building of the next generation of nuclear power stations. In 2008, deep geological disposal of intermediate- and high-level waste (ILW and HLW), which are the most hazardous components of the UK's waste legacy, was adopted as UK Government policy (Department for Environment Fisheries and Rural Affairs *et al.*, 2008). Indeed, ILW dominates (in terms of volume) the UK radwaste inventory at

364,000 m<sup>3</sup>, approaching 80% of the overall total (Table 1). The current UK geological disposal facility (GDF) disposal concept for ILW, a very important wasteform, is predicated on the waste being grouted in stainless steel containers, then backfilled with cement to give a high pH, reducing disposal environment intended to limit radionuclide mobility, based on a generic concept developed by Nirex (Nirex, 2003).

The resulting wasteform will be highly complex and include a high cellulose content, leading to potential generation of organic nutrients (and potentially radionuclide complexants), CO<sub>2</sub> and CH<sub>4</sub>. The high metal content of ILW including steel waste, will also lead to H<sub>2</sub> generation as it corrodes anaerobically. This complicated mix of potential microbial growth substrates, in combination with the presence of local radiation fields that may pose only a sub-

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TABLE 1. UK radioactive wasteforms (NDA, 2007; for further details, see Morris *et al.*, 2011).

Waste type	Volume (m <sup>3</sup> )	Activity (TBq)	Disposal concept
ILW	364,000	$2.2 \times 10^6$	Grouted in stainless steel containers, then backfilled with cement to give high pH, reducing disposal environment.
HLW	1400	$36 \times 10^6$	Glass in stainless steel containers, disposed in Cu canisters. with cast iron inserts and backfilled with compacted bentonite.
Spent fuel	11,200	$45 \times 10^6$	Fuel assemblies packaged in Cu canisters with cast iron inserts and backfilled with compacted bentonite.
Plutonium	3300	$4 \times 10^6$	Not well developed. Probably similar to HLW.
Uranium	80,000	$3 \times 10^3$	Not well developed. Probably similar to ILW.

Note that it is likely that in the UK, the plutonium stockpile will be reused via MOX fuel rather than geologically disposed (Department for Energy and Climate Change, 2011).

lethal dose to the most resistant microorganisms, will over the long periods of time required for storage and disposal, most probably result in the development of microbial communities within the highly heterogeneous waste form. This could, in turn, have major implications for (1) the evolution of biogeochemical conditions within the geodisposed materials; (2) wasteform integrity; and (3) radionuclide mobility in and around the geological disposal facility. However, there are considerable uncertainties around the rate and extent of such microbial processes at high pH, in cementitious ILW.

Microbes have the metabolic diversity to colonize the most extreme environments on Earth, ranging from glaciers (Miteva *et al.*, 2004) to hydrothermal systems (Kashefi and Lovley, 2003), and from the most acidic acid mine drainage sites (Bond *et al.*, 2000) to alkali soda lakes (Mesbah and Wiegel, 2008). They are also well known to contribute to a surprisingly diverse deep biosphere (Parkes *et al.*, 1994), and therefore microorganisms clearly have the potential to colonize geodisposed cementitious, relatively nutrient-rich ILW over geological time periods. After closure, the repository will enter a redox cascade from aerobic conditions that will dominate during GDF construction and any extended operational period, to post-closure anoxia, where a range of electron acceptors will be utilized by specialist anaerobic microorganisms. Although there have been several studies of the microbial ecology of relatively high pH aerobic environments, especially soda lakes,

there is a paucity of information on the utilization of alternative electron acceptors under anoxic conditions, with the notable exception of a handful of studies on alkaliphilic sulfate- and Fe(III)-reducing bacteria at moderately high pH (e.g. Ye *et al.*, 2004; Pollock *et al.*, 2007; Sorokin *et al.*, 2008). This needs to be rectified as anaerobic microorganisms have the potential to degrade organic matter through fermentative pathways (including metal complexants such as isosaccharinic acid (ISA) formed by alkali hydrolysis of cellulose; Glaus and van Loon, 2008). This has significant implications for geodisposal, as utilization of electron donors in microbial metabolism can lead to enzymatic radionuclide reduction and the resultant reductive immobilization of U(VI), Tc(VII) or Np(V) (Lloyd, 2003). Also, the formation of reduced iron or sulfide phases through biogeochemical cycling reactions can provide new reactive biomineral surfaces, commonly at the nano-scale, with high surface areas for radionuclide reduction and/or sorption (Lloyd *et al.*, 2011). Overall, anaerobic microorganisms have, therefore, the potential to alter the evolution pathway for the GDF and thus merit further study.

The aim of this study was to determine the biogeochemical diversity of key microorganisms in a high pH environment analogous to ILW systems, with a particular focus on anaerobic processes. Sediment samples were collected from a site in the Peak District, UK that had been contaminated with high pH residues from lime workings for several decades. Sediments were then incubated anoxically

at a range of alkali pH values, in the presence of added electron donors and a range of electron acceptors (nitrate, Fe(III) and sulfate) to explore the limits and rates of bioreduction in alkali-tolerant communities. The rates of microbial reduction were quantified at a range of alkali pH values, and these data were used alongside thermodynamic calculations to identify the potential upper limits of key microbially relevant redox processes within a GDF.

## Methods

### *Field sampling and microcosm set up, sampling and geochemical analysis*

Sediment samples were collected from an area adjacent to a legacy lime works site at Harpur Hill, Buxton, UK. These surface sediment samples (~20 cm depth) were highly alkaline (pH of the porewater was 11–12) and contained high calcium concentrations, broadly analogous to the conditions expected in a cementitious geological repository environment. The concentrations of selected cations in the porewater were determined using a Perkin-Elmer Optima 5300 inductively coupled plasma atomic emission spectroscopy (ICP-AES) system.

Microcosms were set up anaerobically (under an atmosphere of N<sub>2</sub>) in 100 ml serum bottles using 5 g of the Buxton site sediment mixed with 100 ml of liquid medium. The medium contained 9.4 mM NH<sub>4</sub>Cl, 4.3 mM K<sub>2</sub>HPO<sub>4</sub>, 4 mM NaHCO<sub>3</sub>, 10 ml l<sup>-1</sup> of mineral stock solution (Lovley *et al.*, 1984), 7 mM Na-lactate, 7 mM Na-acetate, 0.025 g l<sup>-1</sup> yeast extract and 15 mM of one of the following electron acceptors: NaNO<sub>3</sub>, soluble Fe(III)-citrate or Na<sub>2</sub>SO<sub>4</sub>. Insoluble Fe(III) oxyhydroxide (ferrihydrite; Lovley and Phillips, 1986) was also used in parallel electron acceptor experiments at an equivalent concentration of 15 mmol l<sup>-1</sup>. For each electron acceptor, triplicate microcosms were set up at pH 10, 11 and 12 (pH adjusted with NaOH), as well as sterile (autoclaved) controls. All microcosms were incubated at 20°C and sampled on a weekly basis, after which pH and Eh measurements were taken using a Cole-Parmer 5990-45 electrode and a Mettler Toledo InLab Redox Micro electrode, respectively. The concentration of Fe(II) in the Fe(III)-supplemented microcosms was determined by the ferrozine assay (Lovley and Phillips, 1987). In selected microcosm samples the concentrations of lactate and acetate were determined using a Dionex DX120 ion exclusion chromatograph,

and the concentrations of nitrate, nitrite and sulfate were determined using a Metrohm 761 compact ion exchange chromatograph.

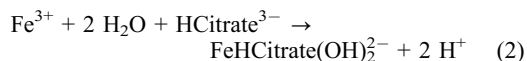
### *Calculation of redox couples*

To complement the experimental investigation into the bioreduction of the different electron acceptors described, the *E'* and  $\Delta G$  of the relevant redox couples were calculated using a fully thermodynamic approach as implemented in *PHREEQCI* (Parkhurst and Appelo, 1999). The *E'* and  $\Delta G$  values for the relevant couples were calculated using the Nernst equation for the relevant pH and electron acceptor concentrations of the experimental set up. Relevant concentrations of species were calculated using the *PHREEQCI* program (Parkhurst and Appelo, 1999). The (SO<sub>4</sub><sup>2-</sup>/HS<sup>-</sup>), (NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>), (NO<sub>3</sub><sup>-</sup>/N<sub>2</sub>) and (N<sub>2</sub>/NH<sub>4</sub><sup>+</sup>) couples were calculated directly within the *PHREEQCI* program using the *Hatches* database (Baston *et al.*, 2007).

The ferrihydrite, Fe(OH)<sub>3</sub>/Fe<sup>2+</sup> couple was calculated using an accepted *E*<sup>0</sup> value of +0.244 V (Stumm and Morgan, 1996) and the concentration of the oxidized and reduced forms were obtained using *PHREEQCI* under the relevant pH conditions; *E'* was then calculated using the Nernst equation:

$$E' = E^0 + \frac{RT}{nF} \log \frac{[\text{Fe}(\text{OH})_3]}{[\text{Fe}^{2+}]} \quad (1)$$

Thermodynamic speciation calculations using the *Hatches* database (Baston *et al.*, 2007) suggested that the dominant 'Fe(III)-citrate' form in solution was the FeHCitrate(OH)<sub>2</sub><sup>2-</sup> species, and the *E*<sup>0</sup> for the reaction given in equation 2 was calculated using log*K* information presented in the *Hatches* database and  $\Delta G_{\text{react}}$  for the standard Fe<sup>3+</sup>/Fe<sup>2+</sup> couple:



For all couples,  $\Delta G_{\text{react}}$  were then calculated using equation 3:

$$\Delta G_{\text{react}} = -nFE \quad (3)$$

## Results

### *Microbial incubations*

Initial investigations of the field samples collected to inoculate the microcosms showed a sediment

system at the Buxton field site that was highly alkaline, with sediment porewaters containing concentrations of  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  of 82, 67 and 29  $mg\ l^{-1}$ , respectively. The pH of the porewaters from the sediments was 11.2, and X-ray diffraction traces from analyses of the sediment phases were dominated by calcite, with halite also being detected (data not shown). These data are consistent with those expected from materials at the legacy lime workings.

When inoculated into anoxic growth medium with a stoichiometric mix of lactate and acetate as electron donors, and a range of electron acceptors, there was a marked difference between the initiation of the relevant bioreduction processes. The final rate of reduction occurred in the order of

nitrate > Fe(III)-citrate > Fe(III) oxyhydroxide > sulfate (Fig. 1). Nitrate reduction was initiated quicker than respiration with any of the other electron acceptors (i.e. within the first week at pH 10), and nitrate was the only electron acceptor that was fully reduced over the course of this study; full reduction occurred within one week at pH 10, and two weeks at pH 11. In both of these cases denitrification was via nitrite, which was detected as an intermediate in the pH 11 microcosms at week one (at concentrations of approximately 12  $mM$ , data not shown). Microbial metabolism in both the pH 10 and 11 cultures resulted in a decrease in pH to 9.5 and 9.7, respectively, and in decreasing Eh values as well. At pH 12, however, there was no evidence of

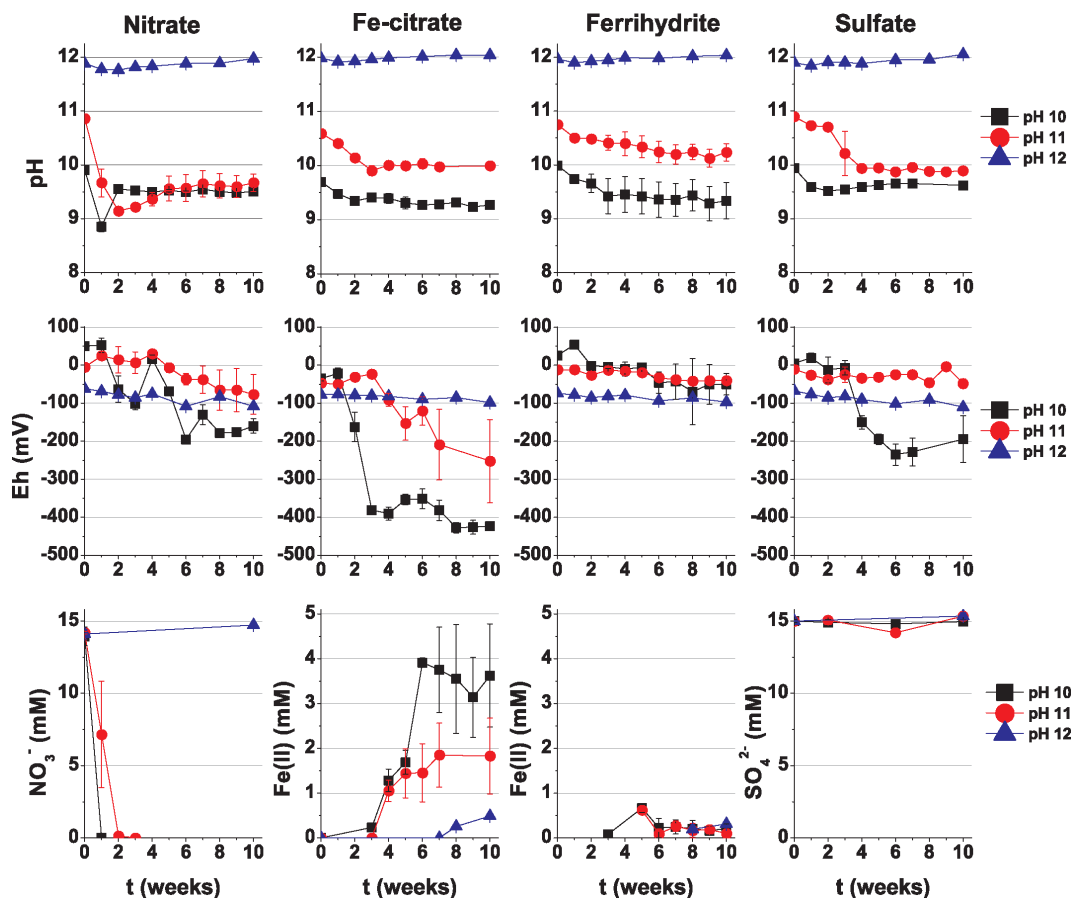


FIG. 1. Measurements of pH/Eh, and  $NO_3^-$ , Fe(II),  $SO_4^{2-}$  concentrations in anaerobic microcosms that were incubated over 10 weeks, and were set up at pH 10, 11 and 12, using lactate and acetate as carbon sources and approximately 15 mM of nitrate, soluble iron (in the form of Fe-citrate), insoluble amorphous Fe(III) oxyhydroxide (ferrihydrite) or sulfate as sole electron acceptors.

nitrate reduction, and no significant change in pH or Eh throughout the experiments suggesting a limit to active bioreduction in this system. There was also no reduction of nitrate in the sterile controls set up alongside these experiments at pH 10 and 11, confirming that the processes observed in the microcosms initially poised at pH 10 and 11 were biologically mediated.

The initiation of microbial Fe(III) reduction in the Fe(III)-citrate supplemented microcosms was much slower than in nitrate-reducing experiments, and the final extent of reduction (measured as ingrowth of Fe(II) in the microcosm slurries) was also lower. Furthermore, it was observed that a precipitate formed at pH 10 and above prior to inoculation, suggesting formation of some insoluble Fe(III) minerals from the strongly chelated Fe(III) added to the medium. Again, microbial utilization of the electron acceptor in these experiments was only noted at pH 10 and 11 (not pH 12). At pH 10, Fe(II) was first detected in the sediments after 4 weeks, and reached 3.9 mmol kg<sup>-1</sup> sediment slurry (26% of the total) by week 6. These changes were accompanied by a marked drop in Eh to -390 mV and a slight drop in pH to approximately 9.5. At pH 11 there was a 2–3 week lag in Fe(III) reduction, with a maximum of 1.8 mmol kg<sup>-1</sup> measured in the sediment slurry after 9 weeks (12% of the total). For the pH 11 systems, again there was a marked decrease in Eh (to -250 mV) and significant decrease to pH 10 over the experimental incubation period. There was negligible formation of Fe(II) at pH 12, and no significant changes in Eh or pH again suggesting a limit to the active bioreduction in this system.

In the enrichments amended with ferrihydrite, the measured reduction of Fe(III) was much less pronounced, even at the lower pH values tested, suggesting restricted use of this form of insoluble Fe(III) as an electron acceptor in this system. There were much lower concentrations of Fe(II) throughout (between 0.2 and 0.6 mmol kg<sup>-1</sup> sediment slurry) and the Eh values in the ferrihydrite systems did not reach those noted in the pH 10 or 11 enrichments amended with the more bioavailable Fe(III)-citrate. Sulfate reduction could not be quantified during the 10 week incubation period at all pH values tested, but there was visual evidence of sulfide formation at pH 10 through blackening of the sediment, which is consistent with a small fraction of the sulfate being reduced, and a significant drop in Eh from about 34mV to -200 mV. Furthermore, sulfate

reduction in the pH 10 microcosms was confirmed after extended (20 weeks) incubation, when sulfate concentrations decreased from 15.33 to 13.60 mM (data not shown).

### *Thermodynamic calculations*

To help identify any thermodynamic criteria that would limit microbial reduction processes at high pH, a series of calculations were conducted to identify the Gibbs free energy changes ( $\Delta G$  kJ mol<sup>-1</sup>) and Eh values associated with the four principle redox couples used in our experiments. All concentrations and redox values were calculated using a thermodynamic approach, and as such kinetically controlled reactions such as the precipitation of ferrihydrite were represented as the thermodynamically stable phase. Clearly, this may not be the same precipitate that forms within our experiments, for which true thermodynamic equilibrium may not have been attained. Similarly, the most thermodynamically stable chemical speciation predicted in the model may differ from that produced under the constraints of the experimental conditions. Finally, the assumption of the Nernst equation (used in our calculations) is that the concentration of the species is equivalent to the activity, which for these low ionic strength systems is a good approximation. In terms of iron speciation, the citrate ligand was by far the strongest complexant in the system that we modelled. Acetate (added in significant excess as the electron donor in the system) had minimal effect on the iron speciation. Modelling results also suggested that at the highest pH values (where little or no experimental evidence for bioreduction was observed), Fe(II) species such as Fe(OH)<sup>+</sup>, Fe(OH)<sub>2</sub><sup>3-</sup> started to dominate with respect to the uncomplexed Fe<sup>2+</sup> ion.

The change in  $\Delta G$  kJ mol<sup>-1</sup> for all couples of relevance to this study are shown in Fig. 2, plotted across a pH range between 7 and 12.5. As expected, of the couples directly studied in the experimental section, the lowest  $\Delta G$  kJ mol<sup>-1</sup> (highest potential energy yield) corresponded to the NO<sub>3</sub><sup>-</sup>/N<sub>2</sub> then the NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> couples, followed by that for the FeHCitrate(OH)<sub>2</sub><sup>2-</sup>/Fe<sup>2+</sup>, ferrihydrite/Fe<sup>2+</sup> and finally SO<sub>4</sub><sup>2-</sup>/H<sub>2</sub>S couples. There was a gradual upward trend for  $\Delta G$  kJ mol<sup>-1</sup> with pH for all couples confirming that there was a lower potential energy yield for these reactions as pH increased. The data for the Eh values for the couples show the expected trend

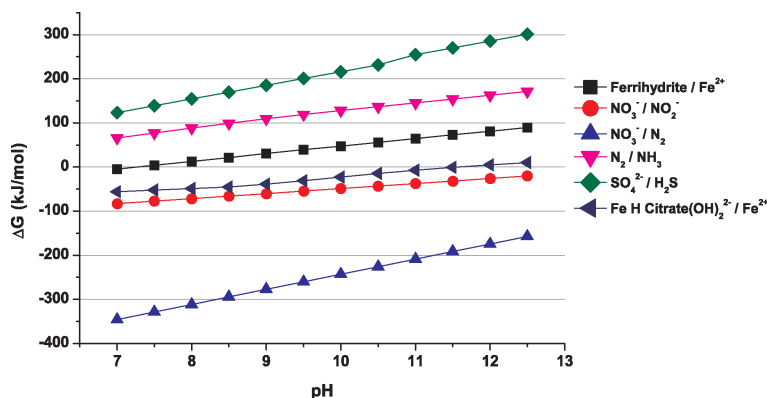


FIG. 2. Calculated Gibbs free energy yield for key biogeochemical redox couples from pH 7 to 12.5.

of  $\text{NO}_3^-/\text{N}_2 > \text{NO}_3^-/\text{NO}_2^- > \text{FeHCitrate}(\text{OH})_2^{2-}/\text{Fe}^{2+} > \text{ferrihydrate}/\text{Fe}^{2+} > \text{SO}_4^{2-}/\text{H}_2\text{S}$  reflecting the cascade of terminal electron accepting processes (Fig. 3). As expected, there was a marked decrease in calculated Eh (and therefore available energy) as the pH increased, especially for the iron species, resulting in their crossing other couples at high pH values. Overall this suggests that the energy yields for Fe(III)-reduction may be very low at high pH.

## Discussion

Using a naturally alkaline sediment as an inoculum, a series of anaerobic enrichment cultures or ‘microcosms’ were set up at pH 10,

11 and 12 to explore the limits of bioreduction of a range of electron acceptors of relevance to ILW geodisposal, under anoxic conditions. Lactate and acetate were used as the electron donors in order to stimulate the extant, presumably alkali-tolerant microbial communities. The experimental results indicated a clear succession in the utilization of electron acceptors, in accordance with published data at near neutral pH (Madigan *et al.*, 2008) and with calculated free energy yields and Eh values over the pH range of interest (Figs 2 and 3). The reduction of nitrate, present in many nuclear waste materials (Lloyd and Macaskie, 2000), via nitrite, was most rapid and went to completion within 2 weeks at pH 10 or 11, which is consistent with the highest free energy yields. This was

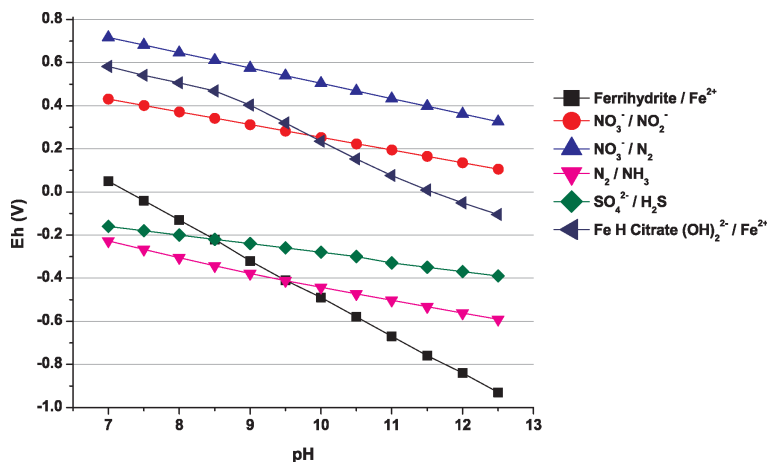


FIG. 3. Calculated Eh values for key biogeochemical redox couples from pH 7 to 12.5.

followed by progressively weaker Fe(III) reduction and sulfate reduction. The reduction of two forms of Fe(III) was tested, and the systems augmented with more bioavailable Fe(III)-citrate, where the Fe(III) was partially complexed by the citrate ligand, was reduced far more efficiently at pH 10 and 11 than the systems augmented with insoluble, amorphous Fe(III) oxyhydroxide (ferrihydrite). This is comparable to data at neutral pH, where Fe(III)-citrate is soluble and can diffuse into microbial cells (von Canstein *et al.*, 2008). Nevertheless, at higher pH this situation is likely to be more complicated as citrate complexation is expected to compete with hydrolysis and precipitation of Fe(III)-oxyhydroxides and consistent with our experiments where we observed some precipitation of amorphous Fe(III) phases in citrate systems raised to pH >9. Overall, this suggests that even with citrate (or other complexants) present, bioavailability of Fe(III) may be a limiting factor due to hydrolysis dominating as pH increases. For ferrihydrite augmented experiments the extremely low solubility of ferrihydrite even at neutral pH, means that it is considered less bioavailable. As expected, it was reduced less efficiently in our experiments at pH 10, 11 and 12, where presumably hydrolysis and precipitation reduces the bio-availability of the Fe(III) even further. This was most probably compounded by the expected transformation of ferrihydrite to more crystalline phases such as hematite at high pH (Shaw *et al.*, 2005). These types of crystalline iron oxides are considered highly recalcitrant in Fe(III)-reducing cultures (Cutting *et al.*, 2009). Further to this, a range of Fe(III) and Fe(II) minerals will form in ILW via the corrosion of steel wastes, and even at these extremely high pH values, the presence of strong chelating agents in the waste itself or formed through alkali lysis of cellulose at high pH (e.g. isosaccharinic acid, ISA; Glaus and van Loon, 2008) may enhance complexation of Fe(III). This will presumably increase the availability of Fe(III) for bioreduction although these processes are poorly constrained at present. Indeed, other experiments from our laboratory suggest that the products of microbial Fe(III) reduction at high pH in these systems, are dominated by magnetite. Bio-magnetite is expected to exert a profound impact on the solubility of redox-active radionuclides including Tc(VII), U(VI) and Np(V) via indirect reductive transformations (Lloyd and Macaskie, 2000; Lloyd *et al.*, 2000, 2002; Lloyd, 2003; McBeth *et al.*, 2011).

Although the enzymes responsible for the bioreduction of Fe(III) in these systems also have considerable potential to reduce a broad range of redox-active radionuclides via direct enzymatic transformations, again the potential for these processes at high pH is poorly constrained (Lloyd, 2003). Sulfate reduction, which sits at the bottom of the microbial anoxic redox cascade studied here, has the lowest potential energy yield at all pH values and was reduced very inefficiently over the timescales studied. Clearly much longer term experiments are warranted to assess the potential impact of this form of anaerobic respiration in ILW. If biogenic sulfide minerals do form over extended time periods, they can also exert considerable control on the solubility of a range of radionuclides (e.g. Lloyd *et al.*, 1998; Moyes *et al.*, 2000).

Although the reduction of many of the electron acceptors studied here is thought to generate alkalinity under more conventional incubation conditions, here we noted a slight drop in pH in our experiments, perhaps due to the formation of acidity generating CO<sub>2</sub> from the metabolism of lactate, acetate and other forms of carbon naturally present in the sediments. Also, at pH 12, there was no evidence for the microbial reduction of any of the electron acceptors tested. Several factors should be considered here: firstly, the microcosms were inoculated with sediment from a natural system at pH ~11.2, a pH value significantly below pH 12. Thus, it is unlikely that microorganisms adapted to such high pH values would be present in the inoculum. Secondly, our experiments were only incubated for a relatively short period of time. During geodisposal scenarios, which are relevant over periods of thousands to several hundreds of thousands of years (rather than weeks to months), there is ample opportunity for microbial communities to adapt to *in situ* conditions, which initially will include pH values >11. Finally, the data that we present here supports the observations of others that the limit for many of the processes we have studied is approaching pH 12. For example, although isolated microorganisms have been reported to be able to grow at pH values up to 12.5 (Takai *et al.*, 2001), this has proved highly contentious (Sorokin, 2005), as this would require a cytoplasmic pH of 10, which is thought prohibitive for microbial metabolism. Microbial growth has been observed, however, up to pH 11.4 in pH-controlled chemostats (Sorokin *et al.*, 2001). In fact, the thermodynamic data presented

here, when compared to physiological models for nitrate and Fe(III) reduction are surprisingly consistent, if we assume that alkaliphilic organisms use similar biochemical mechanisms to those of neutrophilic organisms. This is not unreasonable, as alkaliphiles generally maintain their cellular metabolism at near neutral pH (Horikoshi, 1999). For example, although there is no accepted conserved mechanism of microbial Fe(III) reduction, the involvement of low redox potential *c*-type cytochromes in the outer membrane or periplasm are implicated in most model Gram-negative Fe(III)-reducing bacteria studied to date (most notably *Geobacter* and *Shewanella* species; Lloyd, 2003). The mid-point redox potentials of these heme-containing proteins is typically in the 0 to  $-200$  mV range (Lloyd, 2003), which would make reduction of Fe(III) at pH 12 unlikely using these enzymes (predicted Eh values at pH 12 are  $<-500$  mV for both forms of Fe(III) studied here). For the  $\text{NO}_3^-/\text{NO}_2^-$  couple, values around  $+200$  mV are predicted at pH 12, and here we are very close to or below various estimates for the redox couple of Mo(VI)/IV in the bis-molybdopterin guanine dinucleotide cofactor that is thought to play a role in the active site of nitrate reductase enzymes such as NAR and NAP (Anderson *et al.*, 2000; Potter *et al.*, 2001; Richardson *et al.*, 2001). Thus, nitrate reduction via nitrite may also be unexpected at such high pH values. However, even in high pH cementitious ILW, there will undoubtedly be microniches that are poised at lower pH values which could support microbial metabolism, including the reduction of nitrate, Fe(III) and sulfate. Furthermore, it is clear that with evolution of the geological disposal facility, the pH will evolve to pH 10–11 and this pH will dominate over geological timescales (Morris *et al.*, 2011). Therefore, it is likely that this highly heterogeneous wastef orm has the potential to support a wide range of microbial processes, even at ‘bulk’ pH values beyond those that can theoretically support those processes. Indeed the evolving geodisposal facility will probably have bulk pH values of  $>13$  to  $10^4$  years, then 12.5 up until  $10^5$  years and then approximately pH 10 over  $10^5$ – $10^6$  years after closure. This suggests that microbial processes will be possible in the ILW over the same time periods as the half-lives of long-lived radionuclides, such as Tc and U, that will be released from the wastef orm, emphasizing the importance that these processes are considered in any geodisposal safety case.

## Conclusions

Aerobic metabolism at high pH has been studied intensively in previous studies in a range of high pH environments, and has the clear potential to support colonization of a cementitious ILW backfill in the UK GDF prior to, and just after closure. This study also suggests that a subsequent robust redox cascade will be expected, certainly in environments with pH values up to and including 11. This will include processes mediating the microbial reduction of nitrate, Fe(III) and potentially sulfate. The organisms involved in these processes have the potential to limit the mobility of many redox-active radionuclides that may be present in the wastef orms (including Tc(VII), U(VI) and Np(V)) via direct and indirect redox transformations or by utilizing chelating agents such as ISA produced abiotically under repository conditions as growth substrates. They also have the potential to control gas formation in the repository, another significant component of the long-term safety case for the GDF. They could do this by utilizing hydrogen, formed from the anaerobic corrosion of Fe(0), as an electron donor for anaerobic respiration. They could also minimize the formation of biogenic methane, which could cause over-pressurization of the repository, by keeping methanogenesis in check through substrate (electron donor) competition, if sufficient alternative electron acceptors are present. Thus, this study shows the considerable potential of alkaliphilic anaerobes to colonize and influence the evolution of the high pH environments, representative of cementitious ILW. More targeted biogeochemical work on ILW systems is now warranted, and these experiments should run both with radionuclides and over extended periods of time to determine more accurately the potential rate and extent of such processes in real wastef orms and their impact on radionuclide solubility.

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## References

- Anderson, L.J., Richardson, D.J. and Butt, J.N. (2000) Using direct electrochemistry to probe rate limiting events during nitrate reductase turnover. *Faraday*



- Discussions*, **116**, 155–169.
- Baston, G., Heath, T. and Hunter, F. (2007) *Summary of additions and amendments to the HATCHES chemical thermodynamic database 2002–2007*. Serco Assurance Report for UKNIREX number SA-ENV-0934.
- Bond, P.L., Smriga, S.P. and Banfield, J.F. (2000) Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. *Applied and Environmental Microbiology*, **66**, 3842–3849.
- Cutting, R.S., Coker, V.S., Fellowes, J.W., Lloyd, J.R. and Vaughan, D.J. (2009) Mineralogical and morphological constraints on the reduction of Fe(III) minerals by *Geobacter sulfurreducens*. *Geochimica et Cosmochimica Acta*, **73**, 4004–4022.
- Department for Energy and Climate Change (DECC) (2011) *Management of the UK's Plutonium Stocks*. URN 11D/819, Dec 2011. Department for Energy and Climate Change, London.
- Department for Environment Fisheries and Rural Affairs (DEFRA), Department for Business, Enterprise and Regulatory Reform (BERR) and the Devolved Administration for Wales and Northern Ireland. (2008) *Managing Radioactive Waste Safely: A Framework for Implementing Geological Disposal*. DEFRA, London, 100 pp.
- Glaus, M.A. and Van Loon, L.R. (2008) Degradation of cellulose under alkaline conditions: new insights from a 12 years degradation study. *Environmental Science & Technology*, **42**, 2906–2911.
- Horikoshi, K. (1999) Alkaliphiles: some applications of their products for biotechnology. *Microbiology and Molecular Biology Reviews*, **63**, 735–750.
- Kashefi, K. and Lovley, D.R. (2003) Extending the upper temperature limit for life. *Science*, **301**, 934–934.
- Lloyd, J.R. (2003) Microbial reduction of metals and radionuclides. *FEMS Microbiology Reviews*, **27**, 411–425.
- Lloyd, J.R. and Macaskie, L.E. (2000) Bioremediation of radioactive metals. Pp. 277–327 in: *Environmental microbe-metal interactions* (D.R. Lovley, editor). ASM Press, Washington DC.
- Lloyd, J.R., Nolting, H.F., Sole, V.A. and Bosecker, K. (1998) Technetium reduction and precipitation by sulfate-reducing bacteria. *Geomicrobiology Journal*, **15**, 45–58.
- Lloyd, J.R., Sole, V.A., Van Praagh, C.V.G. and Lovley, D.R. (2000) Direct and Fe(II)-mediated reduction of technetium by Fe(III)-reducing bacteria. *Applied and Environmental Microbiology*, **66**, 3743–3749.
- Lloyd, J.R., Chesnes, J., Glasauer, S., Bunker, D.J., Livens, F.R. and Lovley, D.R. (2002) Reduction of actinides and fission products by Fe(III)-reducing bacteria. *Geomicrobiology Journal*, **19**, 103–120.
- Lloyd, J.R., Byrne, J.M. and Coker, V.S. (2011) Biotechnological synthesis of functional nanomaterials. *Current Opinion in Biotechnology*, **22**, 509–515.
- Lovley, D.R. and Phillips, E.J.P. (1986) Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. *Applied and Environmental Microbiology*, **52**, 751–757.
- Lovley, D.R. and Phillips, E.J.P. (1987) Rapid assay for microbially reducible ferric iron in aquatic sediments. *Applied and Environmental Microbiology*, **53**, 1536–1540.
- Lovley, D.R., Greening, R.C. and Ferry, J.G. (1984) Rapidly growing rumen methanogenic organism that synthesizes coenzyme M and has a high affinity for formate. *Applied and Environmental Microbiology*, **48**, 81–87.
- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2008) *Brock Biology of Microorganisms*, twelfth edition). Pearson/Benjamin Cummings, San Francisco, California, USA.
- McBeth, J.M., Lloyd, J.R., Law, G.T.W., Livens, F.R., Burke, I.T. and Morris, K. (2011) Redox interactions of technetium with iron-bearing minerals. *Mineralogical Magazine*, **75**, 2419–2430.
- Mesbah, N.M. and Wiegel, J. (2008) Life at extreme limits – the anaerobic halophilic alkalithermophiles. Pp. 44–57 in: *Incredible Anaerobes: From Physiology to Genomics to Fuels* (J. Wiegel, R.J. Maier, and M.W.W. Adams, editors). Blackwell Publishing, Oxford, UK.
- Miteva, V.I., Sheridan, P.P. and Brenchley, J.E. (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. *Applied and Environmental Microbiology*, **70**, 202–213.
- Morris, K., Law, G.T.W. and Bryan, N.D. (2011) Geodisposal of higher activity wastes. Pp. 129–151 in: *Nuclear Power and the Environment* (R.M. Harrison and R.E. Hester, editors). Issues in Environmental Science and Technology, **32**. Royal Society of Chemistry, Cambridge, UK.
- Moyes, L.N., Parkman, R.H., Charnock, J.M., Vaughan, D.J., Livens, F.R., Hughes, C.R. and Braithwaite, A. (2000) Uranium uptake from aqueous solution by interaction with goethite, lepidocrocite, muscovite, and mackinawite: an X-ray absorption spectroscopy study. *Environmental Science & Technology*, **34**, 1062–1068.
- Nirex (2003) *Generic Post-closure Performance Assessment*. Nirex Report N/080.
- Nuclear Decommissioning Authority (2007) *UK Radioactive Waste Inventory*. Nuclear Decommissioning Authority, Moor Row, Cumbria, UK.

- Parkes, R.J., Cragg, B.A., Bale, S.J., Getliff, J.M., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J. and Harvey, S.M. (1994) Deep bacterial biosphere in Pacific Ocean sediments. *Nature*, **371**, 410–413.
- Parkhurst, D.L. and Appelo, C.A.J. (1999) *User's guide to PHREEQC (version 2) – A computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations*. U.S. Geological Survey Water-Resources Investigations Report 99-4259.
- Pollock, J., Weber, K.A., Lack, J., Achenbach, L.A., Mormile, M.R. and Coates, J.D. (2007) Alkaline iron(III) reduction by a novel alkaliphilic, halotolerant, *Bacillus* sp. isolated from salt flat sediments of Soap Lake. *Applied Microbiology and Biotechnology*, **77**, 927–934.
- Potter, L., Angove, H., Richardson, D. and Cole, J. (2001) Nitrate reduction in the periplasm of Gram-negative bacteria. *Advances in Microbial Physiology*, **45**, 51–112.
- Richardson, D.J., Berks, B.C., Russell, D.A., Spiro, S. and Taylor, C.J. (2001) Functional, biochemical and genetic diversity of prokaryotic nitrate reductases. *Cellular and Molecular Life Sciences*, **58**, 165–178.
- Shaw, S., Pepper, S.E., Bryan, N.D. and Livens, F.R. (2005) The kinetics and mechanisms of goethite and hematite crystallization under alkaline conditions, and in the presence of phosphate. *American Mineralogist*, **90**, 1852–1860.
- Sorokin, D.Y. (2005) Is there a limit for high-pH life? *International Journal of Systematic and Evolutionary Microbiology*, **55**, 1405–1406.
- Sorokin, D., Tourova, T., Schmid, M.C., Wagner, M., Koops, H.P., Kuenen, J.G. and Jetten, M. (2001) Isolation and properties of obligately chemolithoautotrophic and extremely alkali-tolerant ammonia-oxidizing bacteria from Mongolian soda lakes. *Archives of Microbiology*, **176**, 170–177.
- Sorokin, D.Y., Tourova, T.P., Mussmann, M. and Muyzer, G. (2008) *Dethiobacter alkaliphilus* gen. nov. sp. nov., and *Desulfurivibrio alkaliphilus* gen. nov. sp. nov.: two novel representatives of reductive sulfur cycle from soda lakes. *Extremophiles*, **12**, 431–439.
- Stumm, W. and Morgan, J.J. (1996) *Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters*, third edition. John Wiley & Sons, Inc., New York.
- Takai, K., Moser, D.P., Onstott, T.C., Spoelstra, N., Pfiffner, S.M., Dohnalkova, A. and Fredrickson, J.K. (2001) *Alkaliphilus transvaalensis* gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. *International Journal of Systematic and Evolutionary Microbiology*, **51**, 1245–1256.
- von Canstein, H., Ogawa, J., Shimizu, S. and Lloyd, J.R. (2008) Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. *Applied and Environmental Microbiology*, **74**, 615–623.
- Ye, Q., Roh, Y., Carroll, S.L., Blair, B., Zhou, J.Z., Zhang, C.L. and Fields, M.W. (2004) Alkaline anaerobic respiration: isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. *Applied and Environmental Microbiology*, **70**, 5595–5602.