Microbial Reduction of Fe(III) under Alkaline Conditions Relevant to Geological Disposal

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To determine whether biologically mediated Fe(III) reduction is possible under alkaline conditions in systems of relevance to geological disposal of radioactive wastes, a series of microcosm experiments was set up using hyperalkaline sediments (pH ~11.8) surrounding a legacy lime working site in Buxton, United Kingdom. The microcosms were incubated for 28 days and held at pH 10. There was clear evidence for anoxic microbial activity, with consumption of lactate (added as an electron donor) concomitant with the reduction of Fe(III) as ferrihydrite (added as the electron acceptor). The products of microbial Fe(III) reduction were black and magnetic, and a range of analyses, including X-ray diffraction, transmission electron microscopy, X-ray absorption spectroscopy, and X-ray magnetic circular dichroism confirmed the extensive formation of biomagnetite in this system. The addition of soluble exogenous and endogenous electron shuttles such as the humic analogue anthraquinone-2,6-disulphonate and riboflavin increased both the initial rate and the final extent of Fe(III) reduction in comparison to the nonamended experiments. In addition, a soluble humic acid (Aldrich) also increased both the rate and the extent of Fe(III) reduction. These results show that microbial Fe(III) reduction can occur in conditions relevant to a geological disposal facility containing cement-based wasteforms that has evolved into a high pH environment over prolonged periods of time (>100,000 years). The potential impact of such processes on the biogeochemistry of a geological disposal facility is discussed, including possible coupling to the redox conditions and solubility of key radionuclides.

Many nations have now decided that radioactive wastes, including intermediate-level radioactive wastes (ILW) from civil nuclear power programs, will be disposed of in deep geological disposal facilities (GDFs) (1). In the United Kingdom, the current GDF concept comprises ILW encapsulation typically in cementitious grout and steel canisters prior to emplacement in the GDF between 200 and 1,000 m below the surface (1). After the operational lifetime of the facility, the current generic plan for United Kingdom ILW involves backfilling with cementitious materials. Regardless of the backfill material, it is clear that the grout within the ILW waste form and the engineering of any GDF will result in a significant amount of cementitious materials in many different GDF designs. In the current generic United Kingdom model for ILW, with cementitious backfill, it is expected that resaturation will result in the generation of an alkaline plume from reaction with the cementitious materials in the disposal facility. This alkaline plume will evolve with time as the chemical make-up of the repository is altered by reaction with deep groundwater (2). Furthermore, the alkaline fluids will react with the surrounding environment to form a hyperalkaline chemically disturbed zone that persists over tens to hundreds of thousands of years (1).

The repository will be exposed to air during the operational lifetime of several tens of decades. After closure, microbiologically mediated anoxia is predicted to develop as oxygen is purged from the repository by chemical and presumably biological processes. Indeed, biogeochemical processes are potentially important in geological disposal, but very little work has explored anaerobic redox cycling pathways at high pH. The ILW waste packages are likely to contain elevated levels of organics such as cellulose, which have been found to degrade into a range of electron donating substrates under alkaline conditions (3). Both corrosion of the steel drums and radiolysis of groundwater are expected to liberate hydrogen gas which may also serve as a potent electron donor for anoxic microbial communities (4). In terms of electron accepting processes, the ILW materials will undoubtedly contain Fe(III) from oxic corrosion of wasteforms and iron used in engineering (e.g., rock bolts), and Fe(III) may form a significant part of the geosphere surrounding the disposal facility.

Microbially mediated metal reduction is ubiquitous in both natural and engineered environments (5, 6), and Fe(III) reduction can lead to gross changes in iron speciation, with the precipitation of mixed Fe(II)/Fe(III)-bearing phases such as magnetite and Fe(II) phases such as siderite or vivianite all possible end products. There are a wide variety of prokaryotes that are able to respire Fe(III) at circumneutral pH (6), with the best-studied examples being Geobacter (7) and Shewanella (8) species, and microorganisms such as these may exist in localized, neutral pH microniches within the pH 13 waste form. However, in the United Kingdom generic ILW system, after closure, resaturation will produce a hyperalkaline leachate where the initial pH is expected to be >13, with pH 10 to 11 conditions expected to occur in the chemically disturbed zone for several tens to hundreds of thousands of years. Clearly, under these high-pH conditions and adequate concentrations of suitable electron donors and electron acceptors, the de-
velopment of microbial communities within the waste and host rock may be promoted. There is, however, a paucity of information about the extent and type of microbial processes operating under such conditions. Natural hyperalkaline Ca$^{2+}$-rich groundwater systems formed by the serpentization of primary silicate materials olivine and pyroxene, have been studied for their microbial diversity (9). Furthermore, a select range of alkaliphiles capable of Fe(III) reduction have also been isolated from natural (10–13) and anthropogenic (14, 15) sites with pH values from 9.5 to 12.9. However, the impact of these microorganisms on materials and processes related to ILW remain essentially unknown.

Humic substances, both naturally occurring and anthropogenic derivatives from the waste form, may influence the biogeochemistry in radioactive waste disposal through their ability to act as both ligands for radionuclides (16, 17) and as "electron shuttles" for extracellular redox processes such as Fe(III) reduction. In terms of impacts on Fe(III) reduction, shuttling electrons via semiquinone moieties alleviates the need for direct microbe-mineral surface interactions, increasing the rates of Fe(III) reduction (6, 18, 19). In addition, secreted electron shuttles, including flavin compounds such as flavin mononucleotide and riboflavin produced by Flavobacterium sp., have also been shown to accelerate the rate of Fe(III) reduction (20). Microbially mediated Fe(III) reduction also affects the speciation of key long-lived radionuclides such as U (21), Tc (22), and Np (23) under ambient conditions. Reduction can be enzymatic (24, 25) or due to abiotic electron transfer reactions with products of microbial reduction (23, 26), for example, mediated by Fe(II)-bearing minerals (5). Thus, it is critical to understand the potential for microbially mediated biocycling processes under conditions relevant to geological disposal facilities. Here, we explore microbially mediated Fe(III) reduction in a model alkaline system chosen to be of relevance to intermediate level waste disposal. Specifically, we have explored the reduction of Fe(III) oxyhydroxides added to near surface alkaline sediments taken from a legacy lime working site in the Peak District, Derbyshire, United Kingdom, and determined the effect of microbially reduction on iron mineralogy at pH 10 in this system. Overall, our aim was to assess the scope for bioreduction processes by indigenous microbial populations in sediments from an alkaline geological environment. In turn, this will inform the significance of such anaerobic microbial processes in the safe geological disposal of alkaline, intermediate level wastes.

MATERIALS AND METHODS
Near surface sediment (~20-cm depth) was collected adjacent to legacy lime workings at Harpur Hill Buxton, United Kingdom. Sediment was placed into sterile plastic containers and stored in the dark at 4°C. Sediments were dominated by calcite, quartz, and ankerite. Surface waters were at pH ~11.8 and were dominated by Na$^+$, K$^+$, and Ca$^{2+}$ with measurable Sr$^{2+}$ and silicon. For microcosm experiments, ferrihydrite was synthesized using methods outlined by Cornell and Schwertmann (27, 28).

Microcosm experiments. To investigate the impact on indigenous microorganisms on high pH sediments, microcosms were prepared, in triplicate, in sterile serum bottles with Buxton sediment slurried with surface waters (solid solution ratio 1:5). The sediment slurry was then supplemented with excess Fe(III) as ferrihydrite (120 mM) to provide Fe(III) as an electron acceptor for anaerobic growth. This allowed exploration of biogeochemical processes at a pH known to allow microbial reduction to develop in these sediments (2) and under conditions expected to be relevant to deep geological disposal. The following variations were tested: (i) an oxic control, (ii) Buxton sediment slurry at pH 10 groundwater with no added electron donor ("oxic, no electron donor"), (iii) Buxton sediment slurry at pH 10 with 10 mM sodium lactate and 5 g of yeast extract liter$^{-1}$ (0.5% [wt/vol]; "oxic, electron donor"), (iv) Buxton sediment slurry at pH 10 with electron donor and 100 mM anthraquinone-2,6-disulfonate (AQDS) ("oxic AQDS"), (v) Buxton sediment slurry at pH 10 with electron donor and 2 mg ml$^{-1}$ of a natural source of Elliot Lake humic acid (ELHA; "anoxic ELHA"), and (vi) Buxton sediment slurry at pH 10 with electron donor and 2 mg of Aldrich humic acid ml$^{-1}$ added ("anoxic AHA"). The Elliot soil humic acid was purchased from the International Humic Substances Society (15102H), and Aldrich humic acid was purchased from Sigma-Aldrich; in both cases the humic acids were used as received. The bottles were sealed with thick butyl rubber stoppers and the headspace was flushed for 5 min with N$_2$ to create anoxic conditions. The bottles were then incubated at 20°C in the dark. Throughout the incubations, the pH of the microcosms dropped, especially during the first week of incubation, so manual daily pH adjustment of the experiments to pH 10 was achieved with 2 M NaOH. Sample manipulations and analyses were performed under anoxic conditions as appropriate using an aseptic technique.

Geochemical analyses. The pH and the reduction potential (Eh) were measured upon sample withdrawal with a calibrated (pH 7, 10, and 12) Denver Instrument digital meter. Biogenic Fe(II) and total Fe concentrations on sediment slurries were assessed by the ferrozine assay (29). In addition, sediment porewaters were filtered (<0.2-μm pore diameter; Acrodisc) under anaerobic conditions and organic acids, NO$_3^-$, and SO$_4^{2-}$ were analyzed using a Dionex DX120 ion chromatograph.

Mineralogical characterization X-ray (powder) diffraction. At experimental endpoints, mineral residues were removed from the reduction experiments and dried under anoxic conditions. The dried solids were ground into a fine slurry under anoxic conditions with a few drops of amyl acetate and the sample was then analyzed using a Bruker D8 Advance X-ray diffractometer with a Cu Kα1 source. The data were collected at 3° < 2θ < 70° with a 0.02° step size.

TEM. Transmission electron microscopy (TEM) was carried out using a Phillips CM 200 electron microscope at the Leeds Electron Microscopy and Spectroscopy (LEMAS) Centre, University of Leeds, Leeds, United Kingdom. The microscope was equipped with a field emission gun, an energy dispersive X-ray analysis (EDX) detector (Oxford Instruments, ISIS software), and a Gatan imaging filter (GIF200). All images were obtained using an operating beam voltage of 200 kV. In order to confirm the presence of specific mineral phases, selected area electron diffraction (SAED) patterns were acquired using an appropriate diffraction aperture. Prior to introduction in the chamber, aliquots were obtained from each of the anoxic dry powder samples (typically several milligrams) and dispersed in several milliliters of anoxic water using an ultrasonic probe (Misonix; Microson XL) operating at a power of 15 W (root mean square). A droplet of each of the resulting dispersions was placed on a carbon grid (Agar Scientific) and allowed to dry under anoxic conditions before imaging.

X-ray absorption spectroscopy (XAS) and X-ray magnetic circular dichroism (XMCD). XAS spectra at the Fe L$_3$ edge were collected on the Magnetic Spectroscopy beamline 4.0.2 at the Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA. Samples were dried and ground in an anaerobic cabinet and mounted onto carbon tape attached to the sample probe. The sample probe was transported to the beamline in a sealed anoxic container and loaded into the sample chamber under a backflow of N$_2$ to minimize any potential exposure to air. Measurements were made in total electron yield mode using circularly polarized X-rays with an effective probing depth of ca. 3 to 4 nm, with most of the synchrotron energy lost within the first few nm.

XMCD spectra were derived from the difference between two XAS spectra collected under the application of two opposite magnetic fields (±0.6 T), parallel and antiparallel to the beam direction. XMCD spectra are used to obtain information about magnetization, site location, and...
valence state (number of d electrons). Atomic multiplet calculations were fitted to the XMCD to quantify the site occupancies of Fe cations with the crystalline material [i.e., Fe(II) and Fe(III) in octahedral (Oh) and tetrahedral (Td) coordination] (30–32).

Ribosomal intergenic spacer analysis (RISA). DNA was extracted from sediment samples (0.2 g) and microcosm incubations and subsequent enrichment cultures (200 µl) using a PowerSoil DNA isolation kit (PowerSoil DNA isolation kit; MO BIO Laboratories, Inc., Solana Beach, CA). The 16S-23S rRNA intergenic spacer region from the bacterial RNA operon was amplified as described previously using primers ITSF and ITSReub (33). The amplified products were separated by electrophoresis in a Tris-acetate-EDTA agarose gel (1% [wt/vol]). DNA was stained with 4 µl of a 10-mg/ml ethidium bromide solution and viewed under short-wave UV light. Significant changes in microbial community changes identified by band shifts in the RISA justified further investigation by DNA sequencing of 16S rRNA gene clone libraries.

Amplification, cloning, and sequencing of 16S rRNA gene sequences. A fragment of the 16S rRNA gene, ~1,490 bp, was amplified from samples using the broad-specificity primers 8F (Eden 1991) and 1492R (34) using protocols described previously (22). Briefly, TaKaRa ExTaq polymerase (Millipore UK, Ltd., Watford, United Kingdom) was used to amplify DNA from the sample extract according to previously published protocols and cloned into a vector containing topoisomerase I-charged vector arms (Agilent Technologies, Wokingham, United Kingdom) prior to transformation into Escherichia coli competent cells expressing Cre recombinase (Agilent Technologies) (22). White transformants that grew on Luria-Bertani agar containing ampicillin and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) were screened for an insert using PCR, using primers that were complementary to the flanking regions of the PCR insertion site of the cloning vector.

The resulting PCR products were purified using an ExoSap protocol, and nucleotide sequences were determined by the dideoxynucleotide method as described previously (22). Sequences (typically 900 bp in length) were analyzed using Mallard (35) to check for the presence of chimeras or sequencing anomalies. Operational taxonomic units (OTU) were determined at a 97% sequence similarity level using Mothur (36). The individual OTU sequences were analyzed using the sequencing database of known 16S rRNA gene sequences provided on the Ribosomal Database Project (37) to identify nearest neighbors.

Nucleotide sequence accession numbers. The 16S rRNA sequence data were submitted to GenBank under accession numbers JX417189 to JX417369.

RESULTS AND DISCUSSION

Fe(III) reduction. A series of sediment based microcosms were set up primarily to determine the rate and extent of Fe(III) reduction in pH 10 sediment microcosms amended with ferrihydrite and to probe the Fe(III)-reducing capability of the sediments and associated microbial communities. Oxidizing conditions, indicated by positive redox potential and the presence of predominantly Fe(III) minerals in 0.5 N HCl extracted sediment slurries, were seen in all treatments before incubation and in oxic controls throughout the time series (Fig. 1a). After anoxic conditions were established (indicated by a drop in Eh), minor nitrate present in the starting systems (14 ± 4 µM without electron donor present and 38 ± 2 µM with electron donor) was removed after 3 days in all experiments. To assess the extent of Fe(III) reduction in these systems, a set of sediment slurries were incubated without added electron donor additions. Fe(III) reduction in these controls occurred, with an increase in 0.5 N HCl extractable Fe(II) after day 3 (Fig. 1b). Furthermore, significant increases in Fe(II) after 28 days were observed, coupled to a drop in Eh to −150 mV (Fig. 1b). These values suggest that these high pH sediments contained indigenus, labile organic material, which supported a low level of microbial reduction of Fe(III).

All anoxic microcosms with added electron donor (Fig. 1c to f) showed enhanced microbial reduction, with clear development of Fe(III)-reducing conditions after 3 days, with Eh values dropping and stabilizing at −400 to −500 mV. Therefore, the addition of lactate and yeast extract clearly stimulated the activity of Fe(III)-reducing microorganisms in these experiments, with lactate completely consumed within the 28 day incubation (see Fig. S1 in the supplemental material). Several organic acids were also produced during the incubation; the carbon balance was nonstoichiometric and presumably complicated by the presence of other carbon

FIG 1 0.5 N HCl extractable Fe(II) concentration (□) and redox potential (Eh) (■) in microcosms containing Buxton sediments supplemented with 120 mM ferrihydrite incubated under a range of biogeochemical regimes as defined in Materials and Methods: oxic control (a); anoxic, no electron donor (b); anoxic, electron donor (c); anoxic AQDS (d); anoxic riboflavin (e); and anoxic Elliot Lake humic acid (ELHA) (f).
sources in the yeast extract and/or sediment. In these ferrihydrite-augmented systems, the sulfate concentration was low (37 ± 18 μM) in the anoxic, no-electron-donor system and did not drop over the experimental period. Sulfate concentrations were much higher in the electron-donor-amended systems (469 ± 85 μM), presumably from the yeast extract, but showed no appreciable reduction over the incubational period, suggesting that sulfate reduction had not commenced by 28 days, reflected by the high Fe(III) loading for these systems, and intense competition for the added electron donor, exacerbated by the energetically unfavorable sulfate/sulfide couple at high pH (2).

Interestingly, the addition of the electron shuttles AQDS (Fig. 1d) and riboflavin (Fig. 1e) resulted in the lowest reduction potentials of all the systems tested (−540 ± 3 mV and −552 ± 7 mV, respectively, after 14 days), and rapid development of Fe(III) reduction was observed in both systems. Overall, the highest concentrations of 0.5 N HCl extractable Fe(II) were observed in these systems with 45 ± 1 mM and 44 ± 1 mM 0.5 N HCl extractable Fe(II) at 7 days in AQDS- and riboflavin-amended systems, respectively. The levels of 0.5 N HCl extractable Fe(II) in these systems decreased after 14 days and this, coupled to the fact that the Eh remained significantly reducing over the experimental period (−504 ± 23 mV and −486 ± 20 mV, respectively, after 28 days), suggested that Fe(II) was being sequestered into the a newly formed Fe(II)-bearing mineral. Thus, at the high pH of these systems where metal ions, including Fe(III), will be sparingly soluble [e.g., for Fe(OH)₃, ~10⁻⁶ mol kg⁻¹ (38)], both AQDS and riboflavin enhanced the rate of metal reduction. Presumably, these compounds alleviated the need for direct contact for electron transport between the cell surface and the electron acceptor, thus enhancing the rate of Fe(III) reduction. This suggests that the presence of both natural and anthropogenic electron shuttles, such as humic substances or secreted flavin molecules, respectively, may enhance development of Fe(III)-reducing conditions in the evolved GDF environment.

Interestingly, initial experiments with a natural humic material, Elliot Lake humic, showed no significant increase in the rate of Fe(III)-reduction compared to that noted without the added humic material (Fig. 1c and f). However, under the experimental conditions studied, Elliot Lake humic was clearly poorly soluble at pH 10, forming a black suspension when added, and these results suggest that it was unable to act as a solid state electron shuttle in contrast to recent work on neutral pH systems (39). Further investigations used Aldrich humic acid which was soluble and flavin enhanced the rate of metal reduction. Presumably, these compounds alleviated the need for direct contact for electron transport between the cell surface and the electron acceptor, thus enhancing the rate of Fe(III) reduction. This suggests that the presence of both natural and anthropogenic electron shuttles, such as humic substances or secreted flavin molecules, respectively, may enhance development of Fe(III)-reducing conditions in the evolved GDF environment.

Mineralogical characterization. (i) XRD. To analyze the mineralogical changes associated with microbial Fe(III) reduction, at the end of the experiment, samples of the sediment slurry were analyzed by X-ray diffraction (XRD). Analysis of Buxton sediment with or without ferrihydrite amendment, prior to incubation, showed an XRD spectrum dominated by calcite, presumably as a weathered mineral produced from carbonation of CaO from the lime workings, with minor quartz and ankerite [Ca(Fe, Mg, Mn)(CO₃)₂] similar to other studies at this site (40). Furthermore, 0.5 N HCl extraction with hydroxylamine of the sediments not amended with ferrihydrite indicated that a small but significant proportion of the sediment was present as "bioavailable" Fe(III) (0.3 mg g⁻¹). In the no-electron-donor sediments incubated for 28 days, a broad magnetite peak was present (Fig. 2d), suggesting that this system can support low levels of microbially mediated Fe(III) reduction and forms detectable quantities of magnetite. Indeed, in all end member anoxic systems there was a clear magnetite signal, confirming that microbial processes lead to extensive magnetite biomineralization in these experiments (Fig. 2d to h).

(ii) TEM/SAED. To further characterize the postreduction Fe mineralogy in these experiments, TEM with EDX and SAED was used on the anoxic microcosms amended with added electron donor, both with and without AQDS, at time zero and 28 days. High-resolution TEM images of samples from the ferrihydrite amended oxic sediments showed an iron coating on the calcite-dominated sediments prior to reduction (Fig. 3b). For the bioreduced systems, after 28 days of incubation, individual spherical nanocrystallites in the range of 2 to 10 nm that showed enrichment in Fe and O via EDX and characteristic of biomagnetite were identified (Fig. 3e) (41). Further analysis of the nano crystallites via SAED confirmed the presence of magnetite in the bioreduced system (Fig. 3d) (42).

(iii) XAS/XMCD. Bioreduced minerals were also characterized using Fe L₂,₃ edge spectroscopy to gain a greater understanding of the redox chemistry and potential reactivity of the newly formed iron mineral phase. Magnetite (Fe₃O₄) has a crystal structure consisting of Fe(II) and Fe(III) in octahedral and tetrahedral coordination with a stoichiometric ratio of 1:1:1 [Fe(II)O₆-Fe(III)T₄-Fe(III)O₅], i.e., one-third Fe(II). Prior to incubation, a dried ferrihydrite-amended Buxton sediment sample was analyzed, and the pronounced shoulder feature at 707.5 eV seen in the L₂,₃ edge XAS in the starting material is characteristic of an Fe(III) mineral (Fig. 4, line a) (43). This characteristic Fe(III)-like feature became less prominent in samples taken from the system with electron donor added after the 28-day incubation (Fig. 4, line b) and from the system with an electron donor and AQDS (Fig. 4, line c), which is indicative of an increase in the amount of Fe(II) present. Both end member spectra are characteristic of a biogenic magnetite-like iron mineral phase (43, 44).
The XMCD spectra obtained (Fig. 4) display the characteristic peaks corresponding to Fe(II)O₆, Fe(III)T₆, and Fe(III)O₈ expected for magnetite at both the L₂ and the L₃ edges (43). The significant increase in amplitude of the XMCD signal observed for samples that were incubated with an electron donor corresponds to an increase in the magnetization of the sample, which is another confirmation that a magnetic material is produced by the microbial Fe(III) reduction. The XMCD were fitted to calculated spectra corresponding to Fe(II)O₆, Fe(III)T₆, and Fe(III)O₈ to determine the stoichiometry of the materials (see Fig. S3 in the supplemental material). The results show that the magnetite produced in the absence of an electron shuttle is nonstoichiometric and contains less Fe(II) than expected (0.79:0.99:1.22); however, the magnetite produced with an electron shuttle (AQDS) is much closer to stoichiometry (0.96:0.95:1.08). In comparison, reference biogenic magnetite made using a pure culture of *Geobacter sulfurreducens* and exhibiting a particle size similar to those reported and produced under optimal conditions usually contains a very slight excess of Fe(II) (1.02:0.96:1.01) (45) with respect to the other cations in the crystal structure.

**Microbial communities associated with Fe(III) reduction.** A 16S rRNA gene clone library prepared from the nonincubated Buxton sediment (Fig. 5, column a) revealed a diverse community comprising of 40 different phylotypes from 80 sequences, dominated by two bacterial types from the phylogenetic group *Bacteroidetes*: designated CVCloAm3P11 (27.8%) and CVCloAm3Ph31 (12.5% of the clone library), both of which have been reported previously in Cabeço de Vide, a serpentinization-driven subterrestrial alka-
line aquifer in Portugal (46). After the 28-day incubation in the anoxic system, a far less diverse community was seen, with nine distinct phylogenotypes from 94 sequences analyzed, primarily comprising organisms most closely related to Enterococcus saccharolyticus (97.2% sequence similarity, 48.9% of the clone library), Clostridium ruminantium (100% sequence similarity, 25.5% of the clone library), and Clostridium sp. strain 9B4 (99.6% sequence similarity, 16% of the clone library) (Fig. 5, column b). The dominance of clostridia in these systems may account for the production of propionic and butyric acids during incubation (see Fig. S1 in the supplemental material). Further enrichments in a defined medium adapted from (29), supplemented with ferricyanide as the electron acceptor, and sodium lactate as the electron donor, simplified the community (Fig. S5, column c, and see Fig. S4 in the supplemental material), with close relatives to the following detected: Alkaliphilus oremundii (47) (100% sequence similarity, 30.1% of the clone library), Clostridium sp. strain 9B4 (99.2% sequence similarity, 60.2% of the clone library), Clostridium mangenotii (99.4% sequence similarity, 8.6% of the clone library), and an uncultured bacterium (96% match, 1% of the clone library).

Interestingly, these organisms are Gram-positive bacteria, whom, unlike well-characterized Gram-negative Fe(III)-reducing bacteria (e.g., Geobacter and Shewanella species) (7, 48), lack an outer membrane that contains abundant c-type cytochromes, thought to play a pivotal role in Fe(III) reduction at ambient pH by allowing direct contact with Fe(III) oxides. Little is known about the mechanism of electron transfer to extracellular minerals in Gram-positive bacteria, although recent studies suggest this to be via a direct reductive mechanism governed by surface-localized multi-heme cytochromes (49, 50). Further work to gain an insight into these processes will be the focus of future studies, including exploring the potential of electron transfer between the cell-mineral interface, enhanced by humic materials which can act as electron shuttles.

Conclusions. Here, we show for the first time that indigenous microbial populations present in sediments from a high-pH legacy lime works have the ability to reduce solid-phase ferricyanide to magnetite as the exclusive postreduction iron mineral phase, where the elevated pH in these systems appears to stabilize the biogenic magnetite formed, in agreement with reported pH/Eh stability field calculations (51). The role of organics has also been investigated and is required to stimulate maximal rates of Fe(III) reduction. Within geological disposal environments, organic carbon would be provided from chemical and biological degradation of cellulose materials common in the ILW. Microbial Fe(III) reduction could prove important in utilizing, as electron donors, compounds such as isosaccharinic acid that are formed chemically by alkali hydrolysis and can form strong mobile complexes with radionuclides. Fe(III) reducers could also potentially use hydrogen formed from steel corrosion as an electron donor, and these organisms could therefore act as a sink for reducing equivalents that could otherwise be destined for methanogenesis (4). Thus, microbial Fe(III) reduction, along with a cascade of other redox processes, has the potential to reduce gas production, which would lead to overpressurization and the loss of structural integrity of the emplaced multibarrier system. Since this could promote radionuclide releases, it is a major concern in long-term GDF operation. Interestingly, magnetite is a potent reductant of radionuclides with U(VI) (52), Tc(VII) (53), and Np(V) (23)—all potentially reactive to the biomineral at ambient pH. The findings presented here therefore have clear implications for the safety case that will have to be prepared to underpin the development of any potential GDF designs prior to regulator acceptance.

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