Redox front formation in an uplifting sedimentary rock sequence: An analogue for redox-controlling processes in the geosphere around deep geological repositories for radioactive waste


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ABSTRACT

Subsurface redox fronts control the mobilization and fixation of many trace elements, including potential pollutants such as certain radionuclides. Any safety assessment for a deep geological repository for radioactive wastes needs to take into account adequately the long-term redox processes in the geosphere surrounding the repository. To build confidence in understanding these processes, a redox front in a reduced siliceous sedimentary rock distributed in an uplifting area in Japan has been studied in detail. Geochemical analyses show increased concentrations of Fe and trace elements, including rare earth elements (REEs), at the redox front, even though concentrations of reduced rock matrix constituents show little change. Detailed SEM observations revealed that fossilized microorganisms composed of amorphous granules made exclusively of Fe and Si occur in the rock’s pore space. Microbial 16S rDNA analysis suggests that there is presently a zonation of different bacterial groups within the redox band, and bacterial zonation played an important role in the concentration of Fe-oxyhydroxides at the redox front. These water–rock–microbe interactions can be considered analogous to the processes occurring in the redox fronts that would develop around geological repositories for radioactive waste. Once formed, the Fe-oxyhydroxides within such a front would be preserved even after reducing conditions resume following repository closure.

1. Introduction

Beneath the earth’s surface, sharp redox fronts divide relatively oxidized from relatively reduced groundwater and/or rock (Krumbein and Garrels, 1952; Schwertmann and Murad, 1988). These fronts are formed by natural processes, including weathering, denudation and diagenesis (Carignan and Nriagu, 1985; Williamson and Parnell, 1994; Thomson et al., 1993), and anthropogenic processes such as ingress of air into man-made excavations. Circumstances in which redox fronts may form that are of particular relevance to the present paper are illustrated schematically in Fig. 1.

Examples of such excavations are various kinds of underground repositories for low level radioactive waste (LLW), intermediate level radioactive waste (ILW), vitrified high level radioactive waste (HLW) and spent fuel (SF). These repositories are generally either constructed, or proposed to be constructed, at depths below the surface of between a few tens of meters (LLW and some ILW) and several hundred meters (some long-lived LLW, ILW, HLW and SF). In Japan, it is planned to build a LLW repository at about 100 m depth at Rokkasho in NE Japan. In contrast, it is envisaged that a Japanese repository for HLW will be
constructed at depths of more than 300 m (e.g. NUMO, 2002).

The formation of redox fronts that separate oxidized and reduced rock is expected to occur within the intact porous rock around the galleries of either kind of radioactive waste repository, or along water-conducting fractures that intersect these galleries. The fronts will form due to the oxidation of previously reduced mineral assemblages by oxidizing air and/or water that diffuses from the galleries into the matrix of the surrounding rock (Yoshida et al., 2006a). This process will occur during the period for which the repository is being operated (receiving wastes, being monitored prior to closure, etc.) and in the very early post-closure period. Most probably, conditions within the repository will become reducing within a few years to tens of years following closure, owing largely to reactions within the engineered barriers to radionuclide migration (e.g. corrosion of metals forming waste canisters). Potentially, however, any redox fronts that are preserved in the rock following this return to overall reducing conditions will continue to influence elemental migration (e.g. SKB, 1999). Development of secondary oxides may continue and uptake/sorption on secondary Fe phases (Akagawa et al., 2006) at the redox front.

In subsurface environments, redox processes are also considered to be influenced by geochemical changes probably caused by microbial activity. Understanding such microbial influences on water–rock interactions around waste caverns is particularly important to demonstrate long-term chemical stability (e.g. Anderson et al., 2006) and influences on sorption of radionuclides (Ferris et al., 2000). Hence, such understanding is important for demonstrating the safety of proposed sites for deep geological radioactive waste isolation (e.g. Pedersen, 1997; West et al., 2002). However, there is insufficient understanding of geochemical changes and the long-term behavior of secondary Fe-oxyhydroxides at redox fronts around waste caverns under reducing conditions where any fluxes of oxidants are small. Such processes have to be taken into account to improve the safety case (OECD/NEA, 2004) applicable to the long-term safety assessment of radioactive waste repository sites.

Whereas preservation of these mineralogical redox fronts could be beneficial for a safety point of view, future migration of the natural redox front between shallower oxidizing water and deeper reducing water to the depth of the repository (or greater depth), would be detrimental with respect to safety. Such migration would imply that the conditions in the repository could become oxidizing, thereby enhancing the mobility of certain redox-sensitive radionuclides, such as those of U. In Japan it is possible that a repository might be sited in an area that undergoes future uplift and denudation, resulting in the redox front between shallower oxidizing water and deeper reducing water approaching the repository with the passage of time (e.g. JNC, 2000). It is important that safety assessments are able to take this process into account adequately. To do so requires that the geochemical processes that control the depth of this redox front during uplift and denudation are adequately understood (Fig. 1). There is some evidence from studies outside Japan that several geochemical processes ensure that conditions become reducing rapidly, within a few meters to tens of meters beneath the ground surface (Banwart et al., 1999). However, other studies have demonstrated the possibility that in some aquifers oxidizing conditions may persist to distances of many kilometers along a groundwater flow path from the point of recharge (e.g. Edmunds et al., 1984). Clearly, the exact depth at which the redox front occurs will depend upon a variety of hydrodynamic and geochemical processes, which will be influenced by the lithological characteristics. Thus, it is to be expected that the depth at which the redox front
occurs will change variably during uplift in different rock types. However, there have been few studies of redox fronts developed within actively uplifting rock sequences.

The study presented here aimed to develop a better understanding of the processes that might control the long-term stability of a mineralogical redox front, whether developed around an underground excavation, or naturally beneath the ground surface. A further aim was to determine how such a redox front might influence the migration of trace elements, such as radionuclides, through the geosphere. This paper therefore focuses on clarifying the inter-relationships between the combined abiogenic and biogenic features of redox fronts, and how they contribute to the persistence of Fe-oxhydroxides under reducing conditions in natural systems. In particular, a redox band with Fe-oxhydroxides that can be considered analogous to the redox features that would develop under anoxic conditions after repository system closure is described in detail.

2. Study site

The study focused on a redox front with Fe-oxhydroxides generated at a depth of several meters below the ground surface, along a single fracture under pore water-saturated conditions. The front is developed in the relatively siliceous Akeyo tuffaceous sandstone within a Tertiary sedimentary rock sequence, the Mizunami formation, which occurs in the Tono Basin of central Japan. The area has also been studied during the development of an underground research laboratory (URL) (Fig. 2) to establish a technical basis and methodology for HLW disposal in Japan (e.g. JNC, 2000; Iwatsuki et al., 2005). Within the URL program, a shaft and several boreholes have been drilled to investigate the regional groundwater geochemistry and its evolution (Iwatsuki et al., 2001, 2005). These boreholes have been logged and groundwater samples have been obtained from the Akeyo tuffaceous sandstone.

The Mizunami formation consists of lacustrine to shallow marine sediments with volcanic material and in the study area is up to several hundred meters thick. The age of the studied sediments has been estimated from plant and shell fossils (Kano and Yanagisawa, 1989; Itoigawa, 1993) and fission track dating (Kobayashi, 1989; Sasao et al., 2006), to be about 16 Ma. The facies are mainly fine-grained, compacted massive sandstones, which extend further along the fracture and penetrate from the fracture's surface deep into the rock formation.

The present topography of the Quaternary cover rocks was formed during a period of gradual uplifting within the last several hundred thousand years (Sasao et al., 2006). This time period therefore places an upper limit on the interval during which redox reactions could have been progressing within the Akeyo tuffaceous sandstones.

3. Methods

3.1. Geochemical analysis

Intact rock samples were collected from an outcrop that had been freshly exposed in a road cutting. At the investigated locality, the redox front extended into the fresh rock matrix (Fig. 3). In order to clarify the role of fracturing in the development of the redox front, two transects were sampled: transect A crossed a fracture while transect B was located within the un-fractured rock matrix. Both transects were defined so as to lie at approximately the same distance below the surface. However, groundwater had apparently penetrated relatively deeply along the fracture crossed by transect A. The specimens were taken directly from the outcrop after making small pits at each sampling site by using a portable boring machine. The drilling fluid was Millipore™ “MQ” water. Each rock sample was immediately transferred to a plastic bag filled with N2 gas in order to minimize oxidation and any contamination from other materials and/or possible living microbes.

The specimens were first analyzed for major and trace elements, including REEs (Figs. 3 and 4). To obtain the elemental profile perpendicular to the single fracture surface, an intact block sample 20 cm long, was taken from A. Half of the block was carefully cut into 18 pieces parallel to the redox band structure. The remaining half was then used for microbial analysis.

The 18 subsamples were analyzed for major, minor and trace elements (excluding REE) by XRF, and for REEs by ICP-MS. The XRF analyses were done using a Shimadzu SXF-1200 equipped with a Rh X-ray tube. Glass beads were prepared by fusing mixtures of a portion of each sample and anhydrous Li tetraborate in the ratio of 0.7–6.0 g and 2.0–4.0 g, for major and minor element analyses, respectively. Major elements were calibrated with rock reference samples issued by the Geological Survey of Japan (GSJ): Geochemical Reference Sample Data Base, http://www.aist.co.jp/RIODB/db012/welcome.html. Minor elements were calibrated using the standards of Yamamoto and Morishita (1997). Ferrous iron (FeO) was determined by the 0.01 M-KMnO4 titration method. Ferric iron (Fe2O3) was calculated by subtraction of FeO determined by titration from total Fe determined by XRF.

Rare earth elements were analyzed according to the method by Yamamoto et al. (2005) by inductively-coupled plasma mass spectrometry (ICP-MS; HP4500). Solutions were prepared by digestion of approximately 50 mg samples with 2 mL HClO4 + 4 ml HF, followed by separation of REE with a Dowex 50Wx8 column. The eluted solution
was evaporated to dryness and dissolved in about 20 g 2%-HNO₃.

3.2. Microscopic characterization

Rock samples for geochemical analysis were placed in metal-free plastic tubes (20 mL) containing 10 mL of 5% (v/v) aqueous glutaraldehyde, a fixative for electron microscopy. Qualitative analysis was undertaken by energy-dispersive X-ray spectrometry (EDX), using an EMAX (Super Xerophy), HORIBA Co., Japan with a S-4500, Hitachi Co. electron probe microanalyser. When the samples were returned to the laboratory, they were also thin-sectioned and polished, then dehydrated and embedded in epoxy.

Fig. 2. Simplified geological map showing the study site, the locations of nearby boreholes and outcrops of redox bands. A schematic illustration of the redox band at the study site is also given (modified from Iwatsuki et al., 2005).
resin (Epoxy-154). The resulting sections were subjected to mineralogical observation and electron probe microanalysis (EPMA) to determine the spatial variations in microscopic geochemical characteristics. Chemical mapping and supplemental quantitative analyses have been carried out using a JEOL JXA-8800R electron probe microanalyser with wavelength- and energy-dispersive systems. Beam potentials were kept at 15 kV, and currents at 120 nA. The mineralogical composition of ‘Fe-oxyhydroxides’ was also examined with an X-ray diffractometer (XRD; Multi-flex, Rigaku Co.) using crushed and powered samples with Cu (40 kV/20 mA conditions).

3.3. Microbial analysis

The remaining intact half of the block sample was cut aseptically into four zones (Zones I–IV; as shown in Fig. 3; Transects A and B) that were used for microbial studies. Subsamples were fixed with formalin for total cell enumeration and the rest kept at 4 °C, then stored frozen at −80 °C in the laboratory until DNA extraction. To avoid microbial contamination, sterile disposable gloves and labware were used in handling. Each zone had distinctive Fe₂O₃ concentrations and Fe₂O₃/FeO ratios (Fig. 3). Small blocks were sampled from each zone in order to identify the co-existing microbes and to evaluate the role of the microbes during redox front formation. All these samples were placed in sterilized metal-free plastic tubes under a N₂ atmosphere, in order to prevent contamination. The samples were used as described below for total microbial cell counts and for 16S rDNA analysis to determine the structure of the microbial communities.

Total cell counts were obtained at progressively greater distances into the rock matrix from the fracture surface, in each of the samples from Zones I to IV. The samples for total cell counts were fixed with borate-buffered formalin at a final concentration of 4% w/v. Microbial cells were collected in triplicate by filtration on pre-blackened Isopore filters (Millipore; pore size, 0.2 μm; diameter, 25 mm) and stained with a 0.05% w/v acridine orange solution for...
Elemental profiles across the ‘redox front’ in Transect A (from DP1a to DP17) for selected major (A) and minor (B) elements, from a fracture surface into the rock matrix.

4. Results

4.1. Elemental distribution across the front

The geochemical variations in the four zones (shown as I–IV) of each transect can be characterized by a distinctive combination of Fe$_2$O$_3$ concentrations, Fe$_2$O$_3$/FeO ratios and P$_2$O$_5$ concentrations (Fig. 3, Tables 1 and 2).

In Zone I of Transect A, the Fe$_2$O$_3$ concentration increases with distance from the fracture surface. However, the FeO concentration first decreases then increases over the same interval. This variation also causes the Fe$_2$O$_3$/FeO ratio to increase and then decrease across Zone I. In contrast, across Zone I of Transect B the concentrations of both Fe$_2$O$_3$ and FeO, and the ratio Fe$_2$O$_3$/FeO, show no large variations. Significantly, the absolute concentrations of Fe$_2$O$_3$ and FeO in Zone I of Transect A are higher and lower, respectively, than the concentrations of these constituents in Zone I of Transect B. This result suggests that as a whole, Zone I of Transect A has undergone more oxidation than has Zone I of Transect B. However, the fact that the lowest Fe$_2$O$_3$/FeO ratio in Zone I of Transect A occurs close...
Table 1

Analytical results of samples from the "Transect A". Major elements are expressed in %, and minor elements including rare earth elements are in ppm.

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\(a\) Distance means the distance from the fracture surface in mm.

\(b\) T-Fe₂O₃ denotes total iron as Fe₂O₃. FeO of DP01a, 02 and 04 could not be determined because of disturbance by MnO, then T-Fe₂O₃ by XRF is shown in this table.

\(c\) Ig. Loss shows “loss on ignition” determined by the gravimetric method.
to the fracture surface can be interpreted as a result of mixing between chemically different waters, in proportions controlled by hydrodynamic processes. Chemical variations across the other zones show greater similarities between the two analyzed transects. In each transect, Zone II is distinguished by a relatively high Fe$_2$O$_3$ concentration compared to Zones I and III on either side of it. Furthermore, in each transect the Fe$_2$O$_3$/FeO ratios show a generally decreasing trend from Zone II to Zone III and then Zone IV. Thus, compared to the rock further away from the fracture surface, Zone II is relatively oxidized. It is considered that Zone IV has not been reached by the redox front and is a pre-oxidation zone where the geochemical and microbrial characteristics are at background level. The margin of Zone III that is furthest away from the fracture surface therefore represents the maximum depth of redox front penetration.

In Transect A, the mean Fe$_2$O$_3$/FeO ratio of Zone II is lower than that of Zone I, reflecting the more oxidized character of Zone I. In contrast, in Transect B, the Fe$_2$O$_3$/FeO of Zone II is relatively high, compared to that of Zone I.

From Zone II to Zone IV in Transect A and from Zone I to Zone IV in Transect B, there is relatively little variation in the FeO concentrations. This result implies that variations in the Fe$_2$O$_3$/FeO ratios across these profiles were caused by Fe being transported from elsewhere and then fixed as Fe(III). Across both transects, the P$_2$O$_5$ concentrations were caused by Fe being transported from elsewhere and then fixed as Fe(III). Among both transects, the P$_2$O$_5$ concentrations generally correspond to variations in Fe$_2$O$_3$.

Detailed profiles of major and trace element concentrations (Fe as total Fe$_2$O$_3$, Mn (as MnO), Zn, Ni, Ba, Pb, P (as P$_2$O$_5$), Y and reduced Fe (as FeO)) across the front in Transect A are shown in Fig. 4 and Table 1. The redox front that is characterized by the accumulation of oxides and several trace elements, has migrated into the rock matrix for up to 120 mm from the fracture surface. The concentration profiles of the elements have the following features. Firstly, within the rock matrix a relatively high MnO concentration is identified at a location that is closer to the fracture surface (less than 20 mm) than the band where Fe(II) is most concentrated. Several other elements, notably Zn, Ni, Ba and Y, also have very similar distribution patterns to Mn (Fig. 4A and B). The most Fe-concentrated band was formed between about 50 and 60 mm from the fracture surface, beyond which Fe concentrations decrease to a distance of about 120 mm. The concentration profile of P and part of the concentration profile of Y are also similar to the Fe profile.

The elemental profile shows an intimate relationship between Fe and P within the regions (Zone II) of Fe enrichment (Fig. 4A). This characteristic was investigated in more detail by optical microscopy (Fig. 5A) and electron probe microanalysis (Fig. 5B–D). EPMA X-ray maps (Fig. 5C and D) show that the Fe is concentrated in and around the microscopic pore spaces in the redox front. Microscopically, however, it is shown that P enrichment is surrounded and/or enclosed by Fe-enriched layers which commonly form spherical structures. Within these layers and spheres enriched P concentrations of up to 15 wt% are observed (Fig. 5B).

Fig. 6A and Table 2 show analytical data for REE and the distribution patterns of these elements across the redox front. The REE patterns were normalized with respect to the REE concentrations in sample DP15, which has the lowest REE abundances among the samples from Zone IV. Manganese oxide-rich layers in Zone I have relatively high concentrations of REEs but there is a progressive decrease in concentrations towards HREEs from Gd to Lu. In contrast, the Fe$_2$O$_3$-rich layer in Zone II shows relatively low concentrations of LREE with complementary progressive increases in concentrations towards the HREEs from Gd to Lu. The Lu/Gd ratio after DP15 normalization, (Lu/Gd)$_n$, shows a strong positive correlation with the Fe$_2$O$_3$ content (Fig. 6B).

### 4.2. Mineralogical and microscopic morphological features

The mineralogy of the fine to medium grained tuffaceous sandstone mainly consists of detrital quartz, feldspar and volcanic glass and small amounts of fresh biotite and...
hornblende. Secondary framboidal pyrite grains can also be seen. Almost all of these detrital grains are unaltered, even within Zones I to III, in the area where the redox front developed.

Across the redox front, the Fe–P-enriched areas were investigated by optical microscopy and SEM, supported by EDS. They were found to contain secondary fabrics of presumably microbiological origin (Fig. 7). This sedimentary rock contains many micro-pores that range in size from a few μm to several tens of μm (Fig. 7A and B). Detailed observations of the Fe–P-enriched areas (Zones II and III), revealed structures within the pores that are similar to rod-type microbial cell structures. The pores have been coated by Fe-oxyhydroxides and are sometimes occluded completely by solid Fe-oxyhydroxide aggregations. Fig. 7 shows spherical pellets and their aggregations formed on the irregular surfaces of the pore spaces. Fig. 7B shows sporadically distributed spherical pellets accumulating on the irregular inner surfaces of the micro-pores. A broken Fe-oxyhydroxide pellet contains cell-like structures that may be aggregations of bacteria (Fig. 7C). Highly Fe-enriched flocs with cell-like structures have particles of Fe-oxyhydroxides on their surfaces (Fig. 7C). Direct examination with SEM–EDS also shows that the wall of a cell-like structure contains high concentrations of Fe and Si, suggesting the formation of an authigenic Fe-silicate mineral, most probably a clay mineral (Fig. 7B and C). X-ray diffraction data suggest that the Fe-oxyhydroxides that fill the pores do not show the strong peaks that would indicate crystalline phases, probably due to the crystals being too small and having high degrees of disorder.

4.3. Microbial characterization

Most of the observed microbial cells were rod-shaped (ca 0.5 × 1 μm) or coccoid (1 μm in diameter). Fig. 7C shows the cell morphology under SEM. Total counts of cells within the rock samples from Zones I to IV ranged between $1.3 \times 10^6$ and $4.1 \times 10^6$ cells g$^{-1}$ (Fig. 8), which are higher than those reported previously for the Mizunami formation (Murakami et al., 2000). The concomitant high abundance of both Fe(III) and microorganisms may imply a microbial contribution to Fe(III) accumulation in the generally reducing rock environment.

The microbial assemblages in Zones I–IV were phylogenetically characterized by 16S rDNA sequences. This characterization revealed that different microbial communities are distributed in each zone across the Fe(III) migration front (Fig. 9). A total of 76 clones of the rDNA sequences were grouped into 48 phylotypes. Each zone showed a characteristic presence and combination of clones/phylootypes. Acidobacterial clones, related to the Fe-reducing bacterial genus *Geothrix*, were found in each zone, but were most abundant (three phylotypes from three clones, similarity 93.9–95.1%) in Zone II where there was a high Fe$_2$O$_3$/FeO ratio and P$_2$O$_5$ accumulation. These phylotypes, along with the related phylotypes from Zones I and III, formed a unique cluster in *Acidobacteria*. Verrucomicrobial phylotypes were also found in each zone and were most
types affiliated to Verrucomicrobia phylotypes, similarity 98.1%) were affiliated to the phylum Proteobacteria was mainly present in Zone II. More than 80% of the clones (61/76) and phylotypes (39/48) were present in Zones I, III and IV, while those affiliated to γ-proteobacteria were present in Zones I, II and III. The phylotype ascribed to δ-proteobacteria was mainly present in Zone II. More than 80% of the clones (61/76) and phylotypes (39/48) were most closely related to uncultured bacteria. These results suggest that most of the clones/phylotypes recovered in this study were novel and not fully understood.

5. Discussion

5.1. Process of redox front formation

The hydrogeology and hydrogeochemistry of the area around the study site (Fig. 2) have been investigated extensively in recent years (e.g. Iwatsuki et al., 2005). These studies have demonstrated that groundwater flows almost vertically downwards in the shallower sedimentary rocks, including the Akeyo tuffaceous sandstone. The waters in these sedimentary rocks are fresh and of Na–Ca–HCO3 type at shallower depths, but of Na–HCO3 type at deeper levels. The Na–Ca–HCO3 and Na–HCO3 groundwaters are thought to have evolved as a result of plagioclase and calcite dissolution, combined with cation exchange reactions on clay minerals (Iwatsuki et al., 2001; JNC, 2003).

Arthur et al. (2006) investigated redox conditions in the deeper groundwater system and compared in-situ Eh measurements with theoretical Eh values calculated using analyses of SO2−4, HS−, Fe2+ and Fe3+ made on groundwater samples. They found that in the deeper parts (ca. >50 m) of the same rock formation as that considered in the present study, redox conditions could be explained by equilibrium between SO2−4, HS−, pyrite, Fe2+ and Fe(III)-oxyhydroxides. Furthermore, these deeper groundwater samples were found to be slightly alkaline, with pH between about 7.5 and 8.8.

In view of the hydrogeological context, it is reasonable to expect that oxidizing groundwaters would have moved downwards from the surface in the vicinity of the redox band. By comparison with the results of Iwatsuki et al. (2005) and Arthur et al. (2006) the downward moving groundwaters would become anoxic and progressively more alkaline. The fact that the isolated Fe(III) band is concordant with the surface, which is considered to have formed within the last 105 a, based on K–Ar dating of active faults (Moriyama and Niwa, 1985), suggests that the oxidation probably also occurred during the last 105 a. Possibly, the band is still forming at present, though this cannot be proven from the observations. However, it is necessary to explain how Fe-oxyhydroxides formed at a redox front developed at a depth of several metres below the ground surface, rather than closer to the surface where the most oxidizing conditions are expected to have occurred.

The profile of major and trace elements shows that several elements have migrated during redox front formation. In particular, the almost constant FeO concentrations across Zones II–IV in Transect A, and across Zones I–IV in Transect B, contrast with the variable Fe2O3 concentrations and Fe2O3/FeO ratios across these intervals (Fig. 3). Therefore, the variations in the Fe2O3 concentrations and Fe2O3/FeO ratios cannot be explained by local oxidation of FeO from the rock. Instead, Fe must have migrated into the zones where Fe(III) is now more concentrated. Since Fe(III) is relatively insoluble, Fe most probably migrated as reduced Fe(II)-complexes and was then subsequently oxidized at the locations where Fe2O3/FeO ratios are relatively high. The simplest explanation for the concentration of P and the HREE in the same locations as the Fe is that these elements were all transported together.

The most likely origin for the Fe(II) is the minerals within the rock matrix between the land surface and the redox front. The sedimentary rocks contain small amounts of ferro-magnesian minerals, principally biotite, but also include traces of amphibole. There are also small quantities of pyrite. The leaching of Fe(II) from the silicate minerals could have occurred under acidic, anoxic conditions. The pore water originating in the soil zone possibly had pH between about 4.5 and 5 (cf. analyses of groundwater from nearby
shallow sediments in this area, in Arthur et al., 2006). These conditions would have been caused primarily by relatively high $p$CO$_2$ being developed as a result of plant respiration and the generation of organic acids as a result of organic matter decay. Oxidation of trace pyrite in the rocks nearest to the land surface would also contribute to the development of acidic pore waters. However, as the water originating in the soil zone penetrated to progressively greater depths conditions could have become rapidly anoxic, owing to the action of microbes on organic matter in the high-TOC pore water (Table 3) originating in the soil zone and Akeyo tuffaceous sandstone. A similar redox-controlling mechanism has been well described at the Äspö Hard Rock Laboratory, Sweden (Banwart et al., 1999). Leaching of Fe(II) from silicate minerals would have been enhanced under these acidic anoxic conditions (Schott and Berner, 1983). Potentially, complexing of the Fe with organic ligands originating in the sediments and pore water might also have enhanced Fe migration (Lovley and Anderson, 2000).

The principal objection to this suggestion is that the concentrations of Fe$_2$O$_3$ and FeO are effectively the same in the rock on both sides of the redox front (Fig. 3; Tables 1 and 2). However, if the transported Fe(II) was leached from a sufficiently large volume of rock, then a depletion in Fe content would not necessarily be resolved. This possibility can be illustrated by a simple mass balance calculation. In Transect A, the maximum Fe$_2$O$_3$ concentration is 11.72 wt% (Fig. 3, Table 1). This is equivalent to an added amount of Fe of about 0.12 mol per 100 g of rock, or about

Fig. 7. SEM photomicrograph showing morphological features of microbial pellets and their aggregations identified in the sedimentary pores of the ferric iron band (Zone II and III). (A) Aggregation of microbial pellets within the rock’s micro-pores, (B) SEM images of a microbial pellet encrusted by a solid wall with enrichment of Fe, as shown in qualitative analysis by EDS; (C) a separated microbe which has a micro-crystal-like form and Fe-rich aggregates on the cell walls. The composition measured by EDS is also shown.
0.003 mol per cm$^3$ of rock. If this amount of Fe was leached from a 3 m long column of rock (comparable to the present distance between the redox front and the surface) with a 1 cm$^2$ cross-sectional area, then there would be a reduction in Fe content of only about 0.02 wt% in the rock above the redox front.

Although Arthur et al. (2006) did not report data for the very shallow depths considered in the present study, their results indicate that water–rock reactions involving the silicate and carbonate minerals of the rock matrix would lead to the development of alkaline pore water conditions. The mineralogy of the rock matrix adjacent to the redox band, on the side distal from the fracture is similar to that of the deeper rocks sampled by Arthur et al. (2006). Therefore, it is reasonable to suppose that at the depth of the redox band, pore water conditions would become progressively more alkaline at increasing distances from the fracture surface (i.e., along the two profiles from zones I to IV).

Though Fe-silicates may have supplied Fe(II) to the solution where conditions were relatively acidic, within the redox band itself, where conditions were more alkaline and reducing, these Fe-silicates could have remained stable. This stability explains why variations in FeO concentration are not recognized across the band.

![Figure 8](image1)

**Fig. 8.** Total cell counts in 4 rock samples (shown as I–IV of Transect A) distinguished according to the Fe-concentration profile (see Fig. 3). Zones II and III (with high accumulations of Fe(III)) are also characterized by large numbers of microbial cells in the rock matrix, compared to the matrix of Zone IV.

![Figure 9](image2)

**Fig. 9.** Neighbor-joining tree based on 16S rDNA sequences of the sedimentary rock clones (MIZ01 to MIZ48) and the closest species from the zones I–IV as shown in parentheses. Scale bar, 5% estimated evolutionary distance.
The local enrichments in Mn, Zn, Ni, Ba and Y, suggest that these elements have also migrated into the redox front. Most probably all these migrating elements originated in the rock above the zone, and were transported by water infiltrating from the surface. This migration probably occurred by both advection along the fracture surface and diffusion into the rock matrices. Diffusion could have been promoted by precipitation of these elements in oxidized mineral phases, thereby maintaining concentration gradients in the aqueous phase.

A mechanism is needed by which to explain how Fe(II) oxidation could occur under anoxic conditions at a considerable distance below the ground surface. It is also necessary to identify the oxidizing agents that could have been important in the absence of O2 in the siliceous tuffaceous sandstone sequences.

Groundwaters sampled from depths of <50 m in the area have NO3 concentrations up to about 8 mg L−1 (Arthur et al., 2006), whereas deeper groundwaters have very low NO3 concentrations that are generally below the detection limits, which, depending upon the methods used for sampling and analysis, are 0.3–0.02 mg L−1 (Iwatsuki et al., 2005; JNC, 2005; Table 3). The higher levels of NO3 in the shallower waters are likely to reflect agricultural inputs at least in part, since there is some rice cultivation in the wider area (though not close to the redox band). However, the highest NO3 concentrations are still within the range of feasible natural concentrations (cf. World Health Organisation, 2003). Thus, it seems possible that microbially-mediated NO3 reduction may have some role in oxidizing Fe(II) under anoxic conditions. A possible role of NO3-reducing bacteria in the redox reaction is described in the next section.

An alternative possible oxidant is SO4²−, which occurs in significant concentrations in all the groundwaters sampled from the same sedimentary rocks (Iwatsuki et al., 2005; Arthur et al., 2006; Table 3). In the shallow, unconsolidated sediments in this area, SO4²− concentrations are generally a few mg L−1, whereas deeper fresh waters in the sedimentary rocks have SO4²− concentrations that are generally a few tens to hundreds of mg L−1. However, at the depth of the redox band there is no evidence for SO4²−-reducing bacteria (see below), while Fe(II) oxidation coupled to SO4 reduction is not a generally recognized reaction.

It is therefore postulated that Fe(III)-oxyhydroxides in the redox band were precipitated in response to mixing between the acidic, Fe2+-rich water that entered Zone I from the fracture and more alkaline pore water present in Zone IV (which is similar to the deeper groundwater sampled by Arthur et al., 2006). This mixing could have shifted the redox-controlling equilibria in the vicinity of the redox band (Zones II and III), according to the following equation (Appelo and Postma, 1994):

$$10\text{Fe}^{2+} + 2\text{NO}_3^- + 14\text{H}_2\text{O} = 10\text{FeOOH} + \text{N}_2 + 18\text{H}^+$$  \hspace{1cm} (1)

At the relatively low pH of the water in the fracture, this equilibrium would lie to the left. That is, NO3 and Fe2+ could be transported together. However, upon mixing with the more alkaline matrix pore waters, the equilibrium would shift to the right, resulting in the formation of Fe-oxyhydroxides.

It is noted that in groundwater-rock systems where there is pyrite, NO3 preferentially oxidizes the pyrite-S rather than the Fe2+, according to the reaction (Appelo and Postma, 1994):

$$5\text{Fe}^2_2 + 14\text{NO}_3^- + 4\text{H}^+ = 7\text{N}_2 + 5\text{Fe}^{2+} + 10\text{SO}_4^{2-} + 2\text{H}_2\text{O}$$ \hspace{1cm} (2)

However, the increase in pH that would occur during the mixing described above would tend to cause the reaction to lie to the left, potentially leading to the preservation of the pyrite in the rocks around the redox band.

Thus, the formation of the oxidized Fe-minerals is not simply a function of variations in aqueous oxidants and Eh, but also a result of changes in pH. Within the range of redox-pH conditions, there is a very wide stability field for Fe-oxyhydroxides of various crystallinity. The exact location of redox band formation reflected the evolution of all these parameters as a function of mixing (Fig. 10; Table 3).

Why the observed Fe-oxyhydroxides are only poorly crystalline needs to be explained in view of the experiments reported by Pedersen et al. (2005). These workers demonstrated that dissolved Fe²⁺ may catalyze the transformations undergone by Fe-oxyhydroxides under reducing conditions. However, with a pH of 6.5, the experimental solutions were considerably more acidic than the groundwater considered here. As a result, it is to be expected that the recrystallization of the Fe-oxyhydroxides in the redox band would not occur as rapidly as in the experiments.

In summary, the particular location of the redox band is controlled by hydrodynamic processes, principally the relative advective and diffusive fluxes of shallower, low-pH relatively oxidizing water and deeper, higher-pH more reducing water. Greater downward fluxes of the oxidizing
water, and/or smaller fluxes of the reducing water would have caused the redox band to lie at greater depths (Fig. 10). Evidence for this model is provided by the fact that the redox band moves to greater depth in the vicinity of the fracture (Fig. 3). Greater downward fluxes would be expected within a relatively conductive single fracture.

5.2. Role of microbial activity

In addition to the processes described in the previous section, microbial activity might also be necessary to catalyze the redox reactions and to break down any Fe(II)–organic complexes, allowing Fe to be fixed as Fe-oxyhydroxides at the reaction front. Moreover, geochemical analyses, microscopic observation and microbial studies of this sedimentary rock suggest that the microbial activity has contributed to the concentration of Fe-oxyhydroxides at the front. Therefore, the redox front with Fe-oxyhydroxides in the sedimentary rock can be assumed to have two components. Abiotic diffusion may occur at the same time as additional microbial activity leading to Fe(III) precipitation and the accumulation of other elements within the rock matrix.

A process can be proposed in which microbial activity contributes to the accumulation of Fe-oxyhydroxides within a Fe(III) band, which subsequently persists within otherwise reduced host rock. This process is supported by both the microscopic observations and the phylogenetic study of the rock samples from Zones I to IV. In Zone I (reduction), it is suggested that an acidobacterial group related to the Fe-reducing bacterial genus *Geothrix* has a role in the reduction of Fe(III). Due to the action of this microbe, Fe(II) probably becomes more concentrated in the local pore water than in the pore water in the host rock. The resulting concentration gradient and also the infiltration from the surface might have caused Fe(II) migration into the sedimentary sequence as well as along the fracture plane. Additionally, a microbial consortium suggests a continuous redox reaction in Zone II (post-oxidation) and Zone III (oxidation) under anoxic conditions. One of the notable phylogenetic characteristics of the microbial assemblages at the studied site was the dominance in Zone III (oxidation) of the phylotypes affiliated with the phylum *Verrucomicrobia*, which comprises known fermentative bacteria which mediate anoxic production of organic acids (Chin and Janssen, 2002) and acetate (Janssen, 1998). The verrucomicrobial dominance in Zone III (oxidation) is correlated with a possible incipient stage of Fe-oxyhydroxides (Janssen, 1998), as suggested by the increase in Fe3O4/FeO ratio from Zones IV (pre-oxidation) to III (oxidation). For Fe(II)-oxidizing bacteria, Emerson (2000) provides an overview of the research on circumneutral bacterial Fe(II) oxidation as well as the physiology and systematics of currently known Fe-oxidizing bacteria (IOB) (Emerson and Revsbech, 1994a,b). It has been reported that bacterial Fe(II) oxidation may occur in association with micro-scale redox zonation within low-porosity matrix aggregations in subsurface sediments (Hunter et al., 1998). This process may be influenced by anoxic phototrophic (Widdel et al., 1993; Ehrenreich and Widdel, 1994) and chemolithotrophic NO3-reducing bacteria

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A: Akeyo tuffaceous sandstone. MP: multi piezometer monitoring system. PT: pumping test. n.m.: not measured. TC: total carbon. DIC: dissolved inorganic carbon (mainly HCO3-).
(Straub et al., 1996; Benz et al., 1998), or both, to catalyze circumneutral Fe(II) oxidation (Straub et al., 2001).

Straub et al. (2004) also suggest that *Bradyrhizobium* species (z-proteobacteria) have an ability to oxidize Fe(II) with NO$_3^-$ and the ability is widespread among bacteria such as members of the z-, b-, g- and d-subgroup of the Proteobacteria. (Kappler and Straub, 2005). In zones (I–IV) of the redox front taken together, there are a total of 24 clones belonging to *Proteobacteria*, while, particularly in Zone II, a clone with high similarity (99.5%) to *Bradyrhizobium* has been identified. This suggests that NO$_3^-$-reducing bacteria might also have played some role in oxidizing Fe(II) by consuming NO$_3^-$ in the groundwater at the redox front under anoxic conditions. Since most of the Fe(II)-oxidizing NO$_3^-$ reducers isolated so far need an organic co-substrate for growth, i.e. they grow only mixotrophically with Fe(II) (Kappler and Straub, 2005), verrucomicrobial activity is a possible process by which such nutrients can be provided (Jansenn, 1998).

In a study of sediment pore waters Weber et al. (2006) found a lithoautotrophic betaproteobacterium that could oxidize Fe(II). This microbe could also grow heterotrophically in the presence of simple organic compounds, such as acetate, propionate, butyrate, ethanol, pyruvate and succinate, which were the only C and energy sources. Significantly, no growth was observed when NO$_3^-$ was absent. Their results suggest the possibility that relatively small amounts of NO$_3^-$ could have helped to promote the breakdown of Fe(II)–organic complexes that may have facilitated Fe(II) transport. Roden et al. (2004) also showed that Fe(II) oxidation in transitional redox environments has proceeded even when dissolved O$_2$ concentrations in the pore water are low. The interpretation suggested here requires further investigation, but the observations are consistent with the possibility that a redox front characterized by Fe-oxyhydroxides can be formed and subsequently persist under anoxic geological conditions.

5.3. Elemental remobilization and fixation

At the redox front, within the Fe(III) concentration zone, there is also fractionation of Mn. The concentration profile of Mn and other trace elements, Zn, Ni, Ba and Y is apparently explained by co-precipitation of these elements with Mn oxide. The precipitate can be seen as fine opaque grains dispersed through the rock matrix close to the fracture surface. It is the result of an accumulation of Mn from groundwater that is slightly reducing and slightly manganiferous as described above, relatively acid water could have penetrated from the surface and then mixed with deeper, more reduced, Fe(II)-bearing water. Fe-oxyhydroxides then precipitated at the point where the pH– Eh conditions were most favorable. In the profile through Transect A (the fracture), the conditions would be most oxidizing at the fracture surface, where MnO$_2$ precipitates. These oxidizing conditions would also cause the first peak in Fe-oxyhydroxide formation. The precipitation of these minerals may have been an abiological process. Then, slightly further away from the fracture surface, microbial activity produced a micro-environment in which more Fe-oxyhydroxide formed. The lack of almost any observed Fe-depletion in the reduced rock above or below the redox band means that this could not have been the source of the Fe.

Additionally, the fractionation of HREEs from other REEs can be considered to accompany the fractionation of Fe and Mn during migration of the redox front. HREE fractionation can also occur abiotically due to pH variations (Verplanck et al., 2004). The close correlation of HREE enrichment with the deposition pattern of the redox band, however, suggests that microbial activity connected with the redox cycling might also influence their precipitation.

Mass balance calculations were also done using the REE abundance of the redox band and the present formation water (Takahashi et al., 2002). The concentration of HREE in pore water is less than 1 µg L$^{-1}$ in the Akeyo tuffaceous sandstone (JNC, 2003). The highest HREE accumulation across the redox front is several hundred ppm. There is of course a possibility that HREE at relatively high concentrations may have been transported by acidic water from the ground surface (Verplanck et al., 2004). The pH within the Akeyo formation is however more alkaline. A simple calculation shows that, if the band formed in the presence of groundwater with the same REE concentrations as those of the present groundwater, at least 10$^6$ pore-volumes of water would have been required to give the excess REE concentrations. While the time required for such a large volume of water to flush through the rock cannot be estimated precisely, it must have been substantial. An implication is that the microbially-mediated precipitation of Fe(III) in the deeper part of the rock matrix and the consequent concentration of REE continued for a similarly long period of time.

Detailed microscopic examination by SEM–EDS of the Fe(III) in Zones II and III suggests that high microbial activity oxidized Fe(II) to Fe(III) with simultaneous Fe-silica precipitation in almost all the micro pore spaces. This suggests that a microbial community has covered the pore wall with small spherical pellets that mostly represent individual microbial colonies encrusted by Fe-silicates (Fig. 7B and C).

The mechanism involved in Fe-silica precipitation and formation of pellets can be described as a two-step process (Konhauser and Ferris, 1996). Firstly, Fe is electrostatically bound to the anionic polymers of the microbial cell wall, where it subsequently serves as nucleation sites for further metal deposition. In pore waters with relatively high Fe(II) concentration, dissolved Fe will chemically react with those metallic ions already adsorbed onto the cell wall (Konhauser, 1998). A similar process has been reproduced experimentally and has shown that if the silica concentration in the pore water exceeds that of Fe, dissolved silica will be bound preferentially to the cell wall (Urrutia and Beverige, 1994). These findings suggest that the formation of Fe-silicates under low temperature conditions in the redox front is a consequence of bacterial mats and pellets living in a solution carrying Fe and Si.
The morphological features in zones where the redox front had already passed through usually appear to show aggregated masses of pellets, as shown in Fig. 7A and B, suggesting a micro-fossilization preserved by Fe mineralization. Most of the pore spaces were also filled by an aggregation of pellets, suggesting that solid walls have been preserved by Fe mineralization. It seems that the P was concentrated at almost the same time by being assimilated from pore water by bacterial activity (e.g. Konhauser et al., 1994; Southam, 2000). These features also suggest that the binding of silica and phosphate to Fe-oxyhydroxides such as ferrihydrite (Kennedy et al., 2004) may have influenced the fossilization and preservation of the pellets’ solid walls. Close correlations between Fe and P concentrations have been identified previously, for example in the Fe oxide ooid-like structures reported by Schwarz and Ger­mann (1993).

These observations imply a mechanism for long-term elemental fixation and suggest how microbial activity is implicated in the formation of redox bands with Fe-oxyhydroxides.

5.4. Analogues of redox processes in radioactive waste repositories

Features of the redox front studied here are analogous to some characteristics of the redox fronts that would be developed during the post-closure evolution of under­ground excavations such as geological repositories for radioactive waste (whether shallow, for LLW or certain ILW, or deep for ILW, HLW or SF). The extent to which the sorption of radionuclides is affected by secondary Fe-oxyhydroxides is an issue for safety assessment in such cases.

The development of redox fronts and oxide formation is usually unavoidable in radioactive waste repositories, of whatever design. Such a front would progress from the walls of the vaults and tunnels into the surrounding rock matrix, during construction and/or operational phases. This progression would occur particularly where the rock has been damaged by excavation (Nagra, 1999). In such environments, complex redox reactions will be set in train involving the rock, groundwater and the local in-situ or contaminated microbial community as also shown in the Pocos de Caldas’ analogue study (Schorischer and Shea, 1991; West et al., 1990). Iron-oxyhydroxides, major end products of the combined abi­ological and biological redox processes, are progressively built up as the front migrates into the rock. Such a microbial role in the build-up of Fe-oxyhydroxides has been identified around an in situ gallery at the Äspö Hard Rock Laboratory, Sweden (Pedersen, 1997; Anderson et al., 2006).

After a repository has been closed and reducing condi­tions have been re-established, especially when natural groundwater flow resumes, the production of secondary Fe-oxyhydroxides will cease in the area that has been damaged by excavation. However, the fact that abi­otic and biogenic Fe-oxyhydroxides can persist naturally within undisturbed sedimentary rocks under similarly anoxic conditions, suggests that a wide range of secondary Fe-oxyhydroxides might not easily be reduced after a reposi­tory has been closed. Microbial activity may also support the formation of Fe-oxyhydroxides in rock matrices through redox reactions involving Fe-oxidizing and/or Fe-reducing bacteria. The details of how this happens are still little understood. However, such a reaction involving Fe-oxidizing bacteria associated with encrusted Fe-oxyhydroxide nodules that have persisted within reducing sedimentary rock for ca. 10^5 a, has recently been described by Yoshida et al. (2006b, 2007). Similar persistence of Fe-oxyhydroxides has also been demonstrated in laboratory experiments (Roden et al., 2004).

The preservation of Fe-oxyhydroxides in redox fronts that surround closed radioactive waste repositories will influence nuclide retardation by sorption within excavation-damaged rocks (e.g. Bruno et al., 1991). The fact that the Fe-oxyhydroxides are likely to persist means that they will continue to be capable of sorbing many of the nuclides that could potentially be released from the waste long after closure. Thus, it may be too conservative for a safety case to take no account of this sorption, although this has been done almost universally up to now.

The area considered in this study has been uplifted by several hundred meters during the last several hundred thousand years and uplift is thought to be on-going. How­ever, the evidence presented here shows that groundwater conditions could become reducing very close to the ground surface of such an area of uplift. Microbiological activity could well be important in making this change in redox conditions. The availability of organic matter would be important in such circumstances and would prevent the rapid onset of reducing conditions. Future work needs to obtain comparative evidence for redox front migration in a range of different environments (different vegetation cover, uplift rates, rock types, etc.).

6. Conclusions

Detailed characterization of a redox front formed in a reducing sedimentary rock has revealed that Fe(III) defines the visible redox front via an accumulation of Fe-oxyhydroxides in micro-pores and at mineral boundaries. The study has demonstrated that the redox band developed at a depth of several meters below the ground surface within the last ca. 10^5 a. The redox reactions accompanying the formation of the band correspond to a zonation of microbial communities which reflect the scale (mm to m) of variations in the geochemical environment.

Such oxidation fronts could be formed in the deep geo­logical environment surrounding a HLW repository due to ingress of air during the excavation and operational phases. Post-closure, redox fronts may conceivably form by radiolysis of the groundwater caused by the waste (although this is unlikely to be the case where the canister is steel; Alexander and McKinley, 1999). Around shallower repositories for LLW redox fronts may form due to the incursion of near-surface oxidized groundwater. These findings suggest that the matrix is still accessible to contam­inant diffusion and that the redox front interface around an oxidized rock can be an effective site of contaminant retardation. The present study has some general
implications for simulating the migration of contaminants such as radionuclides. It is clearly conservative (worst-case) for such simulations to assume that only Fe-oxyhydroxides immediately adjacent to the excavation walls would effectively retard contaminant migration by sorption. The present study suggests that microbiologically-mediated reactions at some depth within the rock matrix may also add to the ability of the rock to retard contaminants. The formation of Fe-oxyhydroxides at the study site probably required transport of solutes into the rock from the flow path. Such Fe-oxyhydroxides would therefore be at least partially accessible to any migrating contaminants. These data could be useful as an analogue to develop conceptual models of radionuclide retardation in a repository host rock. However, as noted by Hofmann (1999), much more work needs to be carried out on features such as these to ascertain quantitatively the likely efficiency of such ‘retardation effects’ before the data can be integrated into an assessment of long-term repository performance.

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