Effect of extent of natural subsurface bioreduction on Fe-mineralogy of subsurface sediments

Ravi K Kukkadapu*, Nikolla P Qafoku, Bruce W Arey, Charles T Resch and Philip E Long

Pacific Northwest National Laboratory, P O Box 999, Richland, WA 993552, USA

*E-mail: ravi.kukkadapu@pnl.gov

Abstract. Naturally bioreduced zones with considerable sorbed U were recently identified at a former U mining and processing site at Rifle, CO, USA. Most of the sorbed U appears to be associated with Fe minerals. Variably reduced sediment samples were analyzed by suite of techniques, primarily by room temperature Mössbauer spectroscopy. Fe-oxides of different types and crystallinity, and Fe(II)/Fe(III)-containing clays are dominant in all the sediments. The amounts of poorly crystalline Fe(III)-oxide, however, was lower in the reduced samples. In addition, framboidal pyrites with sorbed U were common in the highly reduced sediments. Overall, the information gained from this work may help develop design field strategies for immobilization and stabilization of U(VI) in contaminated subsurface environments.

1. Introduction

The U.S. Department of Energy (DOE) faces the challenge of cleaning up and/or monitoring large, dilute groundwater plumes contaminated by radioactive and hazardous metals, such as U, Tc, Hg, Pu, Cr etc. These contaminants resulted from the legacy of nuclear weapons production at various US-DOE (e.g., Hanford, WA) and metal-mining and ore-processing sites (e.g., Rifle, CO). Various techniques are underway to remediate the contaminated sites with a combination of physical, chemical, and biological methods [1 and references therein]. For example, stimulation of groundwater dissimilatory iron-reducing bacteria (DMRB) is being explored as a potential strategy to reduce soluble U(VI) to insoluble U(IV) at a number of DOE sites including the Rifle site. At Rifle, the addition of acetate (an electron donor) to the subsurface resulted in an initial decrease in U(VI) [2]. The initial loss of U was associated with enrichment of Geobacter sp (a known Fe- and U-reducing bacterium) and production of aqueous Fe(II). Prolonged acetate injection resulted in the longer term stabilization of U, sulfate reduction, and blackening of the sediment, presumably due to precipitation of Fe-sulfide minerals [3].

Iron bearing minerals (both oxides and clays) of the sediment matrix and groundwater chemical composition play an important role in controlling the mobility of aqueous U(VI) [e.g., 4]. Iron oxides such as ferrihydrite, goethite, hematite, and magnetite, and Fe(II)/Fe(III)-containing clays (e.g., smectites, illites) that are ubiquitous in sediments, are good U(VI) sorbents [e.g., 5, 6]. In addition, Fe-sulfides, such as Fe monosulfide (Fe,Ni)S\(_{0.9}\) (mackinawite), greigite (Fe\(_3\)S\(_4\)), and pyrite (FeS\(_2\)) that also exist in the subsurface may be good hosts for U, as well. Laboratory batch studies with pyrite demonstrated that U(VI) adsorption and partial reduction occur on the surfaces of pyrites [e.g., 7, 8].
Naturally reduced zones that are relatively rich in reduced Fe [Fe(II)], sulfide, U(IV), and organic C (OC), compared to oxidized zones, are common at the Rifle site [9]. An understanding of the geo-microbiological aspects of these samples (oxidized and reduced sediments) may thus provide insights into biotic [direct reduction of U(VI) by bacteria] and/or coupled biotic-abiotic processes [bioreduction of Fe(III) followed by abiotic reduction of U by Fe(II)] that may occur under the natural conditions. The objective of this study is to gain insights into the relationship between the extent of natural reduction and associated Fe mineralogical changes on groundwater U attenuation. A series of sediment samples obtained at different locations that reflect varying aquifer conditions was analyzed by various microscopic techniques, $^{57}$Fe-Mössbauer spectroscopy, and chemical extraction methods. The results, for example, biotransformation of native Fe-minerals to Fe-sulfides and their role in U removal, will enhance our understanding of the long-term behavior of U in the groundwater.

2. Materials and Methods

2.1. Sediment Materials

The cores represented a cross section of sediment conditions that ranged from typical aquifer (minimally reduced) to highly bioreduced (within the saturated zone) conditions. The oxidized sample was obtained from a minimally contaminated zone by use of a backhoe (~3 m depth) from an area with relatively low U groundwater contamination (Figure 1a). This sample is designated as RABS (Rifle aquifer background sample). Samples with higher levels of contamination/reduction were obtained from cores drilled in 2007 (Figure 1; expanded scale of the injection gallery is shown in Figure 1b).

![Figure 1: a) Aerial view of Rifle site, CO, with experimental plot highlighted in circle (reduced samples) and square (RABS sample); b) location of samples in the well lay out ([G60 to G-51 are acetate injection wells); U01 and U02 are up gradient monitoring wells; and down gradient wells (D-series)], and c) a core from D08 well (a highly reduced zone) showing the boundary between reduced and oxidized sediments.](image)

The minimally reduced sediment was designated as D05-16 (16 ft sample from well D-05), while the highly bioreduced sample was designated as D08-16 (16 ft sample from well D-08). Transition between reduced zone and unreduced is highlighted in Figure 1c. We focused on the
<53-micron size fractions that contained relatively more sorbed U [6]. The details of size separation and chemical extraction are described elsewhere [6].

2.2. *Scanning Electron Microscopy (SEM)*

For the SEM measurements, polished sections of sediments were carbon coated to make them electrically conductive. The sections were examined using a JEOL 6340f instrument. Images were then collected using a backscattered electron detector for atomic number contrast. A JEOL model 8200 EMPA, with a detection limit of ~100 μg g\(^{-1}\) and optical resolution of 50 nm, was used to take additional measurements including the quantification of U concentration in pyrite.

2.3. \(^{57}\)Fe-\(\text{Mössbauer spectroscopy}\)

Mössbauer spectra were collected at room temperature (RT) using a 50-mCi (initial strength) \(^{57}\)Co/Rh source. The velocity transducer MVT-1000 (WissEL) was operated in a constant acceleration mode (23 Hz, ±12 mm/s). An Ar-Kr proportional counter was used to detect the radiation transmitted through the holder, and the counts were stored in a multichannel scalar (MCS) as a function of energy (transducer velocity) using a 1024-channel analyzer. Data were folded to 512 channels to give a flat background and a zero-velocity position corresponding to the center shift (CS) of a metal Fe foil at RT. Calibration spectra were obtained with a 25-μm-thick Fe(m) foil (Amersham, England) placed in the same position as the samples to minimize any errors due to changes in geometry. The Mössbauer data were modeled with Recoil software (University of Ottawa, Canada) using a Voigt-based structural fitting routine [10]. The coefficient of variation of the spectral areas of the individual sites generally ranged between 1% and 2% of the fitted values.

3. Results and Discussion

3.1. Chemical analysis

The 0.5N HNO\(_3\) acid extractable Fe(II) contents (a measure of extent of reduction) of the oxidized sample/background (RABS) was lower than the reduced samples (D05-16 and D08-16) (Figure 2a); the Fe(II) content of D08-16 is 1.3x D05-16 and 2.5x RABS. Its acid extractable S and U contents (Figure 2b-c), however, was significantly lower than the reduced samples.

\[\text{\text{Figure 2}}: \text{0.5 N HNO}_3\text{ acid extractable a) Fe, b) S, and c) U of each of the three sediments.}\]

The S and U contents of the D08-16 sample are significantly higher (and 10xS and 4xU) than those of the RABS and D05-16 sediments. The D05-16 and RABS sediments have similar U and S compositions. This trend is similar in all of the size fractions (<53-mm, in the present study; 53 to 106 μm and 106 to 149 μm [6]; and >2-mm [9]). These data imply that the D08-16 zone may have
experienced relatively more sulfate-reducing conditions but somewhat similar Fe-reducing conditions. The organic carbon (OC) content of D08-16 is 2-3x higher than that of D05-16, based on analysis of whole sediment [9]. The relatively higher OC content of the zone may have played a role in the extent of reduction.

3.2. Scanning Electron Microscopy

The backscattered electron image of the S-rich D08-16 sediment (highly reduced) is significantly different than the S-poor RABS (oxidized) and D05-16 (minimally reduced) sediments (Figure 3c). Framboidal pyrites are common in the S-rich sediment, unlike in the other two (Figures 3a,b).

![Figure 3](image)

**Figure 3.** Low magnification SEM images of a) oxidized (RABS) showing Ba and S rich particles, b) minimally reduced (D05-16) with Fe, Ba, and S associations (as coatings) framboids, and c) highly reduced (D08-16) sediments showing frequent framboids (within white circles). The approximate atomic elemental composition (major elements) of the bright spots was obtained by energy dispersive x-ray analysis method. High magnification images of two representative framboidal pyrites of different microcrystal morphology and aggregation are shown in Figure 3c (inserts) to emphasize diverse biogeochemical conditions that exist in the zone and their probable effect on framboidal pyrite morphologies.

Framboidal pyrites are spherical or sub spherical aggregates of distinct microcrystals with distinct internal architecture (Figures 3c inserts). The major difference between the RABS and D05-16 sediments, despite similar extractable Fe(II) contents, appears to be in the residence of S; Fe-S-Ba aggregates as coatings (Figure 3b) and incipient Fe-sulfide framboids (not shown) in the D05-16 sample, although not common; while almost all of the S appears to be associated with Ba in the RABS (Figure 3a). Almost the entire sulfide in D08-16 is associated with Fe. The brightness of the spots in the micrographs is due to association of U with framboidal pyrite in D08-16 (Figure 3c), probable association of U with Fe-sulfide in D05-16 (Figure 3b), and Ba alone with or without little U in RABS (Figure 3a).

3.3. $^{57}$Fe-Mössbauer spectroscopy

3.3.1. Oxidized/background (RABS) sediment. Mössbauer spectra of various size fractions were analyzed at different temperatures, but this paper focuses only on the <53-μm-fraction spectrum obtained at RT (Figure 4). The dominant Fe minerals in the sediment are different types of Fe-oxides and clays. From the Mössbauer parameters alone, it is not possible to distinguish the type and relative composition of various clays; muscovite, illite, and clinochlore are identified by x-ray diffraction (XRD) (Figure not included). We couldn’t detect any XRD peaks due to crystalline Fe-oxide(s). Various oxides, such as, hematite (10%), near stoichiometric “well-crystalline” magnetite (11%), and RT-blocked oxide (Al-substituted Fe-oxides); 31%, contribute to the spectrum. In addition to these Fe-oxide phases, substantial amounts of amorphous/superparamagnetic (SP) is
present in this sample, based on a 12-K spectrum of <2-mm size fraction [5]. Thus, the Fe(III) doublet (30%) centered around 0-1.5 mm/sec is a mix of clay Fe(III) and SP Fe-oxide. Finally, magnetite’s Mössbauer features are similar to that of a well-crystalline/large particle (>50 nm sample [11]). Biogenic magnetites, derived from transformation of Fe-oxides by DMRB, are small (5-10 nm) and display a significantly different RT Mössbauer spectrum than that of pedogenic magnetites [12].

3.3.2. Minimally reduced sediment (D05-16). The RT Mössbauer spectrum of the D05-16 sample (Figure 5a) is significantly different from the background (RABS) sample, which is in agreement with the chemical data; it shows a higher extractable Fe/S ratio. The main differences are lower amounts of RT-blocked Fe (22% vs. 31%) and higher central doublet (0 to 1.5-mm/s region) intensity (36% vs. 30%). Thus, the central doublet is a combination peak of clay Fe(III), SP Fe-oxide, and low-spin, diamagnetic Fe-sulfide compounds [pyrite (FeS2) and mackinawite (Fe,Ni)S0.9)]. This is because Fe(II) in these Fe-sulfides and Fe(III) in clay and SP Fe-oxide exhibit slightly different RT Mössbauer parameters, which make it rather difficult to model unambiguously their relative contributions to the spectrum [13]. Partial assignment of the central doublet feature to these Fe-sulfides is in agreement with 1) the presence of Fe-sulfide particles (Figure 3b) and higher

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**Figure 4:** RT Mössbauer spectrum of RABS: Legend: green = hematite; brown = magnetite Fe(III) from octahedral/tetrahedral sites (assuming non-stoichiometry); dark blue = magnetite Fe2.5+ from octahedral sites; red = combination peaks of clay Fe(III) + SP Fe-oxide; blue = clay Fe(II); and grey = RT-blocked Fe-oxide.
Fe/S mole ratio than the oxidized (Figure 2a,b), and 2) indirectly from the derived clay Fe(II)/central doublet intensity ratio. The derived clay Fe(II)/central doublet ratio is lower than the oxidized (RABS), 0.5 vs. 0.6, which is not expected for a reduced clay. Clay Fe(III) is readily bioavailable under field biostimulated conditions [14,15]. Low-temperature applied field Mössbauer studies that are in progress may be useful if they distinguish peaks of these Fe-sulfides from clay Fe(III).

![Mössbauer spectra](image)

**Figure 5:** Room temperature Mössbauer spectra of a) minimally reduced (D05-16) and b) highly reduced (D08-16). Legends are identical to Figure 1 expect the red pattern with lines is a combination peak of clay Fe(III) + SP Fe-oxide + Pyrite.

3.3.3. **Highly reduced sediment (D08-16).** This sample was extensively studied [9]. Fe-oxides (particularly magnetite) and pyrites, which are known U sorbents, are common in this sediment. Extensive reduction affected the Mössbauer spectrum in two ways (Figure 5b). It differs from the RABS and D05-16 sediments mainly in its lower RT-blocked Fe(III)-oxide content (18% vs. 22% of D05-16 and 31% of RABS), and its higher central doublet peak intensity (45% vs. 36% and 30% in D05-16 and RABS, respectively). Lower RT-blocked Fe-oxide content is in agreement with its redox status. Komlos et al., [5] have shown the gradual loss of Fe-oxide [with simultaneous reduction of clay Fe(III)] upon biostimulation. As argued earlier, the lower clay Fe(II)/central doublet ratio is due to accumulation of Fe-sulfides (0.3 vs. 0.5 in D05-16). Framboidal pyrites (and occasional euhedral pyrites) and near stoichiometric magnetites (not included in this study) with surface sorbed U are common in this sediment; U-pyrite interactions are reported in [6]. Fe-S particles with stoichiometry similar to mackinawite are also noted in some micrographs, albeit not common. Thus, the doublet centered around 1-1.5 mm/sec (Figure 5b) is a combination peak of Fe(II) clay, SP Fe-oxide, and predominantly pyrite. The tabulated mole ratio of Fe/S (Figure 2) is 1.8 is significantly higher than that expected if the extracted Fe(II) is coming from Fe-sulfides alone (0.5 in pyrite, and 1 in mackinawite). This discrepancy may be due to extraction of Fe(II) from clays; acid readily dissolves Fe(II) from the reduced clays [14]. Finally, the Mössbauer parameters of the high-spin Fe(II) doublet (assigned to clay in Figure 4) are significantly different from siderite (FeCO₃), a phase anticipated under the described conditions. This assignment is also in agreement with its 12 K Mössbauer spectrum (not included), which is free of siderite sextet; siderite magnetically orders below <35 K [13], and peaks due to griegite (Fe₃S₄) sextet, a metastable intermediate product of mackinwaite transformation to pyrite [16], are absent.
4. Conclusions

An understanding of field biogeochemical process is required to engineer long-term immobilization of groundwater contaminants. Bioavailability/biotransformation of Fe(III)-oxides and Fe(III) in clays and their subsequent role in reduction/attenuation of redox sensitive contaminants, e.g., U(VI), is a key element of this process. The role of the Fe mineral composition of the sediment, groundwater chemistry, and native microbial community composition and dynamics on such transformations would be significant [17]. The comparison of Fe mineralogy of the sediments obtained from various zones—oxidized and from reduced zones that appear to be reduced under Fe-only and/or Fe-and sulfate-reducing conditions—provided insights into the reduction/removal of Fe-oxides and precipitation of pyrite. Sulfate reducing conditions appear to be present in the naturally bioreduced zone and have resulted in loss of Fe-oxide abundance. Precipitation of sulfide apparently plays an important role in sequestering U under these conditions [6] but we also note the possible importance of mixed-valence Fe oxides (e.g. magnetite) in sequestering U. Detailed examination of Fe-U interactions of sediments from column experiments with little or no sulfate in pore water [5] and where reduction of phyllosilicate Fe(III) was noted, may provide additional information on the role of Fe minerals in U attenuation.

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