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THE IMPACT OF BIOSTIMULATION ON THE FATE OF SULFATE AND ASSOCIATED SULFUR DYNAMICS IN GROUNDWATER

Ziheng Miao^{1,2}, Concepcion Carreón-Diazconti³, Kenneth C. Carroll⁴, and Mark L. Brusseau^{1,2,*}

¹Department of Soil, Water and Environmental Science, University of Arizona, 429 Shantz Building #38, P.O. Box 210038, Tucson, AZ, USA

²Department of Hydrology and Water Resources, University of Arizona, Harshbarger Building #11, Tucson, AZ, USA

³Engineering Institute, Autonomous University of Baja California, Mexicali, Baja California, México

⁴New Mexico State University, Las Cruces, NM

Abstract

The impact of electron-donor addition on sulfur dynamics for a groundwater system with low levels of metal contaminants was evaluated with a pilot-scale biostimulation test conducted at a former uranium mining site. Geochemical and stable-isotope data collected before, during, and after the test were analyzed to evaluate the sustainability of sulfate reducing conditions induced by the test, the fate of hydrogen sulfide, and the impact on aqueous geochemical conditions. The results of site characterization activities conducted prior to the test indicated the absence of measurable bacterial sulfate reduction. The injection of an electron donor (ethanol) induced bacterial sulfate reduction, as confirmed by an exponential decrease of sulfate concentration in concert with changes in oxidation-reduction potential, redox species, alkalinity, production of hydrogen sulfide, and fractionation of $\delta^{34}\text{S}$ -sulfate. High, stoichiometrically-equivalent hydrogen sulfide concentrations were not observed until several months after the start of the test. It is hypothesized that hydrogen sulfide produced from sulfate reduction was initially sequestered in the form of iron sulfides until the exhaustion of readily reducible iron oxides associated with the sediment. The fractionation of $\delta^{34}\text{S}$ for sulfate was atypical, wherein the enrichment declined in the latter half of the experiment. It was conjectured that mixing effects associated with the release of sulfate from sulfate minerals associated with the sediments, along with possible sulfide re-oxidation contributed to this behavior. The results of this study illustrate the biogeochemical complexity that is associated with in-situ biostimulation processes involving bacterial sulfate reduction.

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*Corresponding author, brusseau@email.arizona.edu.

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Keywords

sulfate reduction; stable isotopes; isotopic fractionation; iron sulfide precipitation; sulfate mineral dissolution; sulfide re-oxidation

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INTRODUCTION

Approximately 160,000 abandoned hardrock mines are estimated to exist in the 12 western states and Alaska on state, private, or federal lands (GAO, 2008). Of these, approximately 20% are reported to have environmental degradation issues such as surface water or groundwater contamination. An analysis of 156 hardrock mining sites listed or in consideration for listing on the federal Superfund National Priorities List (NPL) as of 2004 indicated that remediation operations will last from 40 years to in perpetuity at a majority of the sites (EPA, 2004). The average cost to remediate a mining site on the NPL is estimated to range from approximately \$20 to \$150 million (Probst and Konisky, 2001; EPA, 2004). Approximately \$5 billion has been expended by federal agencies for cleanup of hardrock mining sites (GAO, 2008), and estimated total costs for the current 156 NPL-related sites range from 7 to 24 billion dollars (EPA, 2004). It is likely that additional sites will require some form of cleanup, with attendant additional costs, given that, for example, 77% of the sites on the Bureau of Land Management's abandoned mine lands inventory require further investigation and/or remediation (BLM, 2013).

Acid rock drainage and its potential impact on surface and groundwater contamination is generally considered to be the primary environmental concern for many hardrock mining sites in the U.S.A. and elsewhere (NRC, 1999, 2002; ITRC, 2008; INAP, 2013; MEND, 2013). Groundwater contamination serves as one of the primary risk drivers for human health exposure at many hardrock mining sites. For example, approximately two thirds of the 129 mining sites currently listed on or proposed for the Superfund NPL or being remediated under the Superfund alternative approach framework have groundwater contamination (EPA, 2013). The most common groundwater contaminants reported for these sites are arsenic, selenium, uranium, sulfate, and heavy metals. A survey of federal and state Superfund mining sites in Arizona revealed that the most common constituents present as contaminants in groundwater are sulfate, arsenic, and uranium, with selenium and perchlorate also present at some sites.

Extensive groundwater contaminant plumes containing constituents such as arsenic, selenium, uranium, and sulfate often form at mining sites because of the relatively high aqueous solubilities of the constituents (in comparison to regulatory standards), their limited retardation (due generally to anionic speciation), and generally low (or very site dependent) attenuation potential. In many cases, the plumes are hundreds of meters to several kilometers long. These large plumes are very expensive to contain and remediate using pump and treat (NRC, 2013), the standard method for treating contaminated groundwater at such sites (EPA, 2002; EPA, 2013).

Developing cost-effective methods to treat mining-impaired waters is deemed a critical research need for remediation of mining sites (NRC, 1999, 2002, 2005; EPA 2004). Very

few alternatives to pump and treat are available for remediation of large groundwater contaminant plumes containing arsenic, uranium, sulfate, or similar constituents. Permeable reactive barriers (PRBs) have been demonstrated to be an effective method for treating waters containing inorganic contaminants (e.g., Hashim et al., 2011; ITRC, 2011) and, for example, can be a robust alternative for controlling acid rock drainage at the source, thereby preventing or reducing contamination of groundwater (e.g., Benner et al., 1997; ITRC, 2011). However, PRBs are impractical for the large, deep contaminant plumes common to mining sites in the western U.S. Monitored natural attenuation (MNA) has become a popular option to consider as a green, lower-cost alternative to pump and treat for metal-contaminated sites (e.g., EPA, 2007a, 2007b). Heavy metals and acidity in acid rock drainage are generally effectively attenuated near the source. Unfortunately, rates of natural attenuation are insufficient to prevent migration of arsenic, uranium, sulfate, or similar constituents at many mining sites (e.g., Moncur et al., 2005; EPA, 2007c), as evidenced by the very large contaminant plumes that typically develop. This is reflected in the fact that MNA is being used at only a very small number of the current Superfund-related mining sites (EPA, 2013). Methods to enhance the rates of natural attenuation and improve the feasibility of MNA for metal-contaminated sites are a current focus of research and development (ITRC, 2010).

In-situ bioprecipitation or biosequestration is one enhanced-attenuation alternative that has potential for remediation of large, deep groundwater contaminant plumes containing arsenic, uranium, selenium, sulfate, and similar constituents (e.g., EPA, 2000; DOE, 2003; Hashim et al., 2011; Miao et al., 2012). This technology entails injection of a reagent solution into the treatment zone to generate reducing conditions, which induces sequestration (via precipitation and/or enhanced adsorption) of the target contaminants. This sequestration in turn reduces bioavailability of the contaminant, thereby reducing risk. A number of laboratory experiments have been conducted to investigate microbially induced precipitation of uranium, arsenic, selenium, and technetium (e.g., Kauffman et al., 1986; Lovely and Philips, 1992; Rittle et al., 1995; Uhrie et al., 1996; Abdelouas et al., 1998, 2000, 2002; Jong and Parry, 2003; Gu et al., 2005; Keimowitz et al., 2007; Teclu et al., 2008; Sun et al., 2009; Kirk et al., 2010; Omoregie et al., 2013). In addition, a small number of in-situ bioprecipitation pilot tests have been conducted at field sites, primarily for uranium (e.g., Anderson et al., 2003; Istok et al., 2004; Wu et al., 2006; Saunders et al., 2008; Williams et al., 2011).

Bacterial sulfate reduction (BSR) is one of the primary processes involved in most in-situ biosequestration efforts. A critical component of BSR processes is the disposition of hydrogen sulfide. Ideally, the sulfide will be fully sequestered via, for example, formation of metal-sulfide precipitates, such as occurs for treatment of acid rock drainage wherein concentrations of heavy metals are typically relatively high. However, concentrations of arsenic, uranium, selenium, etc. are often significantly lower than sulfate concentrations in groundwater contaminant plumes at mining sites, and heavy metals are typically present at only trace levels. Under such conditions, it is likely that iron-sulfide precipitates formed through interaction with indigenous iron oxides associated with the sediment will exert a significant control on sequestration of sulfide (Bottrell et al., 1995; Rickard, 1995, 1997; Knoller and Schubert, 2010) and possibly co-contaminants (O'Day et al., 2004; Saalfeld

and Bostick, 2009; Root et al., 2009). Minimal research has been conducted to evaluate the impact of BSR for systems with higher sulfate and lower metals concentrations, particularly for conditions representative of mining sites in the SW US.

A pilot-scale ethanol-injection test was conducted to investigate the efficacy of biostimulation for remediation of nitrate and sulfate contaminated groundwater at a former uranium mining site (Borden et al., 2012). The objective of the research reported herein is to investigate the impact of the biostimulation test on the fate of sulfate and associated sulfur dynamics. Geochemical and stable-isotope data collected before and after the test were analyzed to evaluate the sustainability of sulfate reducing conditions induced by the test, the fate of hydrogen sulfide, and the impact on aqueous geochemical conditions.

MATERIALS AND METHODS

Site Background

The Monument Valley site is a former uranium mining site located at Cane Valley, Arizona, 24 km south of Mexican Hat, UT (Figure 1). Uranium mining at the site occurred from 1943 to 1968. From 1964 to 1968, batch and heap leaching with sulfuric acid solution was used to process an estimated 1.1 million tons of tailings and low-grade ore at the site (DOE, 1999, 2005). The solutions used in ore processing were discharged to the “new tailings pile”. A groundwater contaminant plume approximately two km long exists at the site, with nitrate and sulfate concentrations as high as 900 mg/L and 2000 mg/L, respectively. Concentrations of heavy metals, uranium, and arsenic are relatively low (maximum ~ 100 µg/L, most values < 10 µg/L) within the study area (Miao et al., 2013a).

The shallow alluvial aquifer that is the focus of this study resides in a north-south oriented paleovalley that is bounded by bedrock on the south and west sides as well as on the bottom. The aquifer is comprised of well-sorted fine to medium sand deposits interspersed with minor fractions of finer silt and clay sediments. Generally, the alluvial deposits range from 1 to 35 meters in thickness, with the greatest depths occurring in the center of the valley. Bedrock is exposed at the southern and southwestern areas of the source zone, and it has been determined that infiltration at these areas serves as the primary source of recharge to the shallow aquifer (DOE, 1999). It is estimated that only a minor fraction of the low annual precipitation recharges the aquifer in the center of the valley (where the plume resides) due to loss from evaporation and plant uptake (DOE, 1999). Depth to groundwater is approximately 11 meters. The screened intervals for most monitoring wells span approximately 15 to 25 m below ground surface (i.e., 10-m screened interval). The mean hydraulic gradient is to the northeast, with a magnitude of approximately 0.01. Hydraulic conductivities range from 0.1 to 5.6 m/day (DOE, 1999).

Background Conditions for Sulfate Concentrations and Isotope Composition

Stable isotope analysis of S and O for sulfate, in combination with geochemical and hydrogeological data, was used recently to characterize the sources and background conditions for sulfate in groundwater at this site (Miao et al., 2013b). Regional baseline concentrations of sulfate in groundwater are low (~20–30 mg/L). Conversely, background

concentrations in groundwater at the study site are elevated (~100 mg/L), which is attributed to the impact of sulfate generation from oxidation of sulfide minerals in the exposed bedrock present in the primary recharge area for the aquifer. The background isotopic composition of sulfate associated with sulfide-mineral oxidation was determined using values obtained for groundwater samples collected from the outcrop/recharge area, which is upgradient of the contaminant source zone and plume. The reported values ($\delta^{34}\text{S} = -11.5\text{‰}$, $\delta^{18}\text{O} = 4.4\text{‰}$) are within the range of sulfate isotope values reported for sulfide oxidation (e.g., Krouse and Mayer, 1999; Mayer, 2005; Schwientek et al., 2008).

Sulfuric acid and associated sulfate salts, formed after neutralization and disposal of the sulfuric acid solutions in the source zone, were determined to be the primary source of the high sulfate concentrations in the groundwater contaminant plume (contributing an estimated ~74% of total sulfate load). The background isotopic composition of sulfate associated with the sourcezone sulfate salts was determined to be $\delta^{34}\text{S} = 24.3\text{‰}$ and $\delta^{18}\text{O} = 12\text{‰}$. These values are within the range of values reported in the literature for sulfate evaporites (Parafiniuk et al., 1994; Krouse and Mayer, 1999; Knoller et al., 2005; Samborska et al., 2013). The isotopic composition of sulfate within the groundwater contaminant plume is expected to reflect the proportional mixing of the two primary sources, as will be discussed below.

Rates of natural attenuation for nitrate and sulfate in the contaminant plume are slow and minimal, respectively, due to the generally oxidative conditions (Carroll et al., 2009; Miao et al., 2013b). Concentrations of organic carbon in groundwater and the sediment are low. Previous laboratory microcosm studies using sediments from the site indicated that ethanol could significantly enhance the rate of denitrification (Jordan et al., 2008; Carroll et al., 2009).

Test Procedures and Analytical Methods

The test area is within the middle of the plume, 550 m downgradient of the source zone (Figure 1). The study area comprises a 3 m $\times$ 12 m plot containing nine monitoring wells (Figure 1, top right). Aqueous concentrations of constituents have been stable at this location for several years. For example, sulfate concentrations for groundwater samples collected from well 765 exhibited a coefficient of variation of 5% during the five years prior to the test (mean = 637 mg/L, st.dev. = 34, n = 9). A preliminary three-day test was conducted a year prior to the primary test, using the push-pull method wherein the solution is injected into well 765, allowed to remain in place for two days, and then extracted (Borden et al., 2012). Examination of monitoring data indicated minimal impact of this test on groundwater sampled from well 729 or those downgradient.

The primary test was conducted using the standard single-well injection method, with well 729 serving as the injection well. Groundwater was extracted from upgradient of the test area and mixed with ethanol to create a 1% ethanol solution, which was stored in a deflatable bladder to maintain anoxic conditions. The solution was injected for 80 hours with a flow rate of 3 L/min. Groundwater samples were collected from all wells at the test plot prior to, during, and after the test. The site was monitored for 200 days after the injection, during which the complete suite of constituents were sampled. Monitoring was

continued after this time at a reduced frequency and focusing only on a few primary constituents. Details of the test methods, as well as the impact of the biostimulation on nitrate are discussed by Borden et al. (2012). The sulfate data and associated sulfur dynamics are discussed herein.

Groundwater samples were collected using dedicated bladder pumps and a QED Micropurge controller. To collect representative samples, wells were purged until field parameters (pH and dissolved oxygen) stabilized, which typically occurred after the removal of roughly two to three borehole-equivalent volumes of groundwater. Measurements of basic field parameters (dissolved oxygen, pH, oxidation-reduction potential, and temperature) were obtained using a flow-through instrument connected to the flow control system. Samples were then collected for nitrogen species, major cations and anions (Na, Mg, K, Ca, Mn, Fe, Cl, and SO₄), ethanol, hydrogen sulfide (H₂S), and sulfate stable isotope ($\delta^{34}\text{S}$) analysis. Samples for nitrogen species were collected in 500-mL HDPE bottles. Samples for major cations and anions were collected in separate 250-mL HDPE bottles. Cation analysis samples were preserved with 1 mL of 1:1 hydrochloric acid (HCl). Both sample sets were stored on ice at 4°C. Finally, samples were collected for sulfate ³⁴S isotope analysis. These samples were filtered using an in-line 0.45 µm Geotech Dispo-a-filter, collected in separate 1-L HDPE bottles, and preserved with hydrochloric acid to pH < 2. The samples were stored on ice in the field then frozen upon returning to the lab to curtail any additional fractionation.

A Hach portable spectrophotometer (Model DR 2800) was used to analyze hydrogen sulfide concentration immediately after sample collection. Major cations were analyzed using a Perkin Elmer ELAN DRC-II ICP-MS (inductively coupled plasma mass spectrometry), following US EPA Method 6020. Major anions (nitrate, nitrite, bromide, and sulfate) were analyzed using a Dionex DX-600 with an AS-40 autosampler, GP-50 gradient pump, and ED-40 electrochemical detector following EPA standard method 300.0. The quantifiable detection limits are 0.1 mg/L for NO₃⁻ and NH₄⁺, 0.5 mg/L for Cl⁻ and SO₄²⁻, approximately 10 µg/L for major cations (Na, K, Ca, Mg, Al), and approximately 1 µg/L for Fe and Mn.

For $\delta^{34}\text{S}$ analysis, sulfate was first precipitated with barium chloride in acid solution. The sulfur isotopes of sulfate were measured for SO₂ gas with a continuous-flow gas-ratio mass spectrometer (ThermoQuest Finnigan Delta Plus XL) at the Environmental Isotope Laboratory of the University of Arizona. Samples were combusted at 1030 °C with O₂ and V₂O₅ using an elemental analyzer (Costech) coupled to the mass spectrometer. Standardization is based on international standards OGS-1 and NBS123, and several other sulfide and sulfate materials that have been compared between laboratories. Calibration was linear in the range -10 to +30 per mil. Precision was estimated to be ± 0.3‰ or better (2 standard deviations), based on repeated internal standards.

Sediment Characterization

Subsurface sediment samples were collected when the boreholes for monitoring wells 741 and 743 were drilled. Several samples from the depth of the screened intervals were analyzed for mineralogy using X-ray diffraction (XRD) and metal content using acid

digestion. The XRD was performed at the Center for Environmental Physics and Mineralogy at the University of Arizona using a PANalytical X'Pert Pro diffractometer. The quantification limit is approximately 1%. The metal content analysis was conducted at the Arizona Laboratory for Emerging Contaminants at the University of Arizona. Samples were digested using 5N nitric acid under microwave treatment. Subsamples of the extractant were analyzed using a Perkin Elmer ELAN DRC-II ICP-MS (inductively coupled plasma mass spectrometry), following US EPA Method 6020. Quantification limits are approximately 10 µg/L for major cations (Na, K, Ca, Mg, Al), 1 µg/L for Zn and Se, 0.5 µg/L for Fe, 0.2 µg/L for Mn, and approximately 0.1 µg/L for the others. Sulfate leaching tests were conducted previously for several sediment samples collected from two boreholes located 500 and 1000 m upgradient of the source zone in an uncontaminated area to establish background conditions (DOE, 1999). Each sample was extracted sequentially using three separate extractants: deionized water, ground water, and 5% hydrochloric acid.

RESULTS AND DISCUSSION

Sediment Characterization

The results of X-ray diffraction analysis for several sediment samples show that the sediment is composed primarily of quartz (82 – 89%), with minor amounts of orthoclase (0 – 4.8%), microcline (0 – 6.7%), albite (3.5 – 5.5%), calcite (0.5 – 2.4%), dolomite (0 – 0.1%), illite (0 – 4.9%), and kaolinite (1 – 2.2%). Iron and manganese contents associated with sediment oxides range from 1267 to 1784 mg/kg and 22 to 36 mg/kg, respectively. These results indicate that the sediment could serve as a source of iron to support potential iron-sulfide precipitation. Organic carbon contents are very low (~0.02%).

The results of sediment-leaching tests conducted by the Department of Energy (DOE) for background (uncontaminated) sediment samples showed water-extractable sulfate concentrations ranging from approximately 180 to 450 mg/kg, with acid-extractable sulfate ranging from 110 to 160 mg/kg. These results indicate that labile sulfate-bearing minerals, unrelated to site contamination, are present in the sediment at the site. Dissolution of these minerals would serve as an additional source of sulfate to the system, which must be accounted for in the interpretation of the biostimulation test results.

Changes in Aqueous Constituents

Sulfate concentrations in groundwater samples collected from the injection well (729) began to decrease soon after injection (Table 1 and Figure 2). Decreases in sulfate concentration were observed after approximately one month for two downgradient wells 730 and 743 (Table 1 and Figure 2). Interestingly, the rates of decrease were greater for these two wells ($k = 0.02 \text{ d}^{-1}$) compared to the injection well ($k = 0.007 \text{ d}^{-1}$). This is most likely because the injection well continuously received fresh groundwater with abundant sulfate from upgradient, whereas the downgradient wells did not (or at least to a lesser extent) due to the sulfate reduction occurring in the vicinity of the injection well. Minor changes (< ~10%) were observed for wells 731, 741, 742, and 744.

Nitrate concentrations decreased to less than 1 mg/L within two weeks for well 729 and remained as such for the entire monitoring period (Table 1). Oxidation-reduction potential decreased from +120 to -220 mV and dissolved oxygen decreased from 2 to 0.2 mg/L after injection of the ethanol solution. Concentrations of dissolved iron and manganese increased from relatively low background levels (~20 µg/L) to 100's of µg/L. The increase in aqueous concentrations of iron and manganese is attributed to the use of iron and manganese oxides as electron acceptors for ethanol transformation, and their resultant associated reduction. Finally, alkalinity increased from approximately 250 to 3000 mg/L as CaCO₃. The increase of alkalinity is attributed to production of HCO₃ from oxidation of ethanol. The changes observed for all of these parameters indicate the development of reducing conditions within the treatment zone. Very similar, but delayed, responses were observed for the downgradient wells 730 and 743 (Table 1 and Figure 2). This indicates downgradient propagation of the reaction zone (reducing conditions).

Disposition of Hydrogen Sulfide

Production of hydrogen sulfide exceeding background was observed coincident with decreasing sulfate concentrations, albeit at concentrations (~20–750 µg/L) significantly below levels stoichiometrically equivalent to the magnitude of sulfate reduction (Figure 2). A dramatic increase in dissolved hydrogen sulfide (>10,000 µg/L) was observed starting approximately 70 days after injection (Figure 2). Similar behavior was observed for all three wells. These high concentrations observed in the latter stage of the test are roughly representative of stoichiometric (1:1) conversion of sulfate to hydrogen sulfide.

The less than stoichiometric-equivalent concentrations of hydrogen sulfide measured in solution during the early stage of the test indicates that a significant fraction of the hydrogen sulfide generated during this period was sequestered or transformed in some manner. There are at least three possible processes that could have served as sinks for sulfide (e.g., Schippers, 2004). One is the formation of iron-sulfide precipitates (Rickard, 1995, 1997; Knoller and Schubert, 2010), wherein the source of the dissolved iron was generated through use of iron oxides as electron acceptors for transformation of ethanol. This process would sequester the sulfide in precipitate form, thereby removing it from solution. A second possible mechanism is microbially facilitated anaerobic oxidation of sulfide with nitrate and/or manganese oxide serving as the electron acceptor (e.g., Burdige and Nealson, 1986; Aller and Rude, 1988; King, 1990; Lovley, 1991). This pathway would transform sulfide, with sulfate as the predominant product. A third possibility is abiotic anoxic oxidation of sulfide by iron and/or manganese (e.g., Burdige and Nealson, 1986; Lovley, 1991; Bottcher and Thamdrup, 2001; Herszage and Afonso, 2003), with formation of sulfide minerals or elemental sulfur depending on extant conditions. These latter two processes proceed under reducing conditions and, thus, the sulfide oxidation can occur simultaneously with continued sulfate reduction.

It is not possible to determine definitively which of these processes was predominant, given that post-test sediment characterization data are not available. However, the relative potential contribution of the three processes can be assessed. Based on the moderate iron content of the sediments, sufficient iron is likely present to support sequestration through the

formation of iron-sulfide precipitates. The results of prior studies involving bacterial sulfate reduction processes at petroleum-contaminated sites have shown that hydrogen sulfide was sequestered as a mixture of iron sulfides and elemental sulfur, with the latter in smaller amounts (e.g., Bottrell et al., 1995; Knoller and Schubert, 2010), indicating the significance of iron-sulfide formation.

The potential impact of microbially facilitated anaerobic sulfide oxidation can be assessed by evaluating its potential based on measured concentrations of relevant electron-acceptor species present in the system. Assuming that hydrogen sulfide is oxidized by Mn oxides to sulfate only (not considering elemental sulfur, which occurs in abiotic oxidation of sulfide), we have $4\text{MnO}_2 + \text{S}^{2-} + 8\text{H}^+ \rightarrow 4\text{Mn}^{2+} + \text{SO}_4^{2-} + 4\text{H}_2\text{O}$. The maximum measured aqueous Mn^{2+} concentration of approximately 4000 $\mu\text{g/L}$ (Table 1 and Figure 2) translates to a maximum oxidation capacity of 0.018 mmol/L of S^{2-} , which is equivalent to 1.7 mg/L sulfate. This concentration of sulfate is negligible compared to its original concentration of approximately 600 mg/L. It is also a small fraction of the final sulfate concentrations measured for groundwater from all three wells, which were generally 20–30 mg/L. Furthermore, nitrate remained at levels below 1 mg/L in the test zone due to denitrification, which limited its potential use for oxidation of sulfide. The abiotic oxidation of sulfide through reduction of iron and/or manganese is also expected to be insignificant given the relatively low iron (~400 $\mu\text{g/L}$) and manganese concentrations (~2000 – 3000 $\mu\text{g/L}$) compared to sulfate concentrations, similar to the calculation above for microbially facilitated anaerobic sulfide oxidation.

Assuming that the formation of iron-sulfide precipitates was the predominant sink for hydrogen sulfide during the first part of the test, it is estimated that the equivalent of approximately 300 mg/L of sulfate was precipitated as iron sulfide in the early stage of the test in the injection zone before hydrogen sulfide concentrations increased significantly. This is based on an assumption that all sulfide equivalent represented by the difference between the amounts of sulfate reduced and sulfide measured in solution occurs as iron-sulfide precipitates. The 300 mg/L estimate translates to approximately 50% percent of the original sulfate in solution. The sequestration of 300 mg/L equivalent sulfate as sulfide requires a release of 15 to 30 mg/kg equivalent of iron from the sediment, assuming all sulfide is precipitated with iron (II) in a mixed form of FeS and FeS₂ (e.g., Knoller et al., 2008). This amount of iron represents approximately 1 – 2% of the total acid-digestible iron measured for the sediment from the study site. Hence, it is likely that the sediment contained sufficient iron to allow formation of iron-sulfides, which supports the hypothesis that such formation was the primary sink for hydrogen sulfide.

A significant increase in dissolved hydrogen sulfide (>10,000 $\mu\text{g/L}$) was observed during the latter stage of the test, as previously noted. The fact that hydrogen sulfide concentrations increased to levels roughly representative of stoichiometric conversion of sulfate would suggest that there was a significant change in conditions that prevented further sulfide sequestration. What this may have been is unclear. Considering iron-sulfide formation as the predominant sequestration process, one possibility is that reducible iron became limiting at that point. While the measured acid-extractable iron content is much greater than that required to support iron-sulfide formation, the results of prior research indicate that only a

portion of total iron associated with iron oxides is reducible (bioavailable), depending upon degree of crystallinity (van Bodegom et al., 2003). Cessation of the formation of iron-sulfide precipitates during hydrogen-sulfide production due to exhaustion of available sediment-phase iron has been observed in prior studies (e.g., Canfield et al., 1989, 1992; Bottrell et al., 1995). The potential of this or other factors cannot be further evaluated in the absence of post-test sediment samples.

Interestingly, significant increases in the concentration of manganese in groundwater (from <1000 to more than 3000 $\mu\text{g/L}$) were observed for the latter stage of the test (Figure 2). The times at which the concentrations begin to significantly increase correspond to the occurrence of significant increases in hydrogen sulfide. It is hypothesized that the increased levels of hydrogen sulfide present in solution (due to decreased sequestration) caused enhanced reduction of manganese oxide (i.e., anoxic oxidation of hydrogen sulfide). These results suggest that the impact of sulfide oxidation increased in the latter stage of the test. However, its influence is likely to have been relatively minor, as discussed above.

Stable Isotope Analysis

The background sulfur isotope composition for sulfate in groundwater in the test plot area was determined by analyzing samples collected from wells 765, 728, 729, 730, and 731 one year prior to the test. An additional set of samples was collected from all wells in the test plot (including the new wells) a few days prior to the test. The mean $\delta^{34}\text{S}$ value is +16.1‰, with a coefficient of variation of 2%. This small variation indicates spatially and temporally uniform conditions were present within the test plot area prior to the electron-donor injection.

The background $\delta^{34}\text{S}$ of the sulfate in uncontaminated groundwater is -11.5‰, as reported above. The $\delta^{34}\text{S}$ of sulfate in the groundwater contaminant plume is different from background due to the impact of sulfate generated from sulfuric acid and associated sulfate salts from mill operation, and reflects the proportional mixing of the two primary sources. The $\delta^{34}\text{S}$ of sulfate for the sulfuric-acid source was determined to be +24.3‰ (Miao et al., 2013b). The value obtained for the baseline $\delta^{34}\text{S}$ of sulfate in the test area of the plume (+16.1‰) prior to the test is consistent with the relative contributions of the two sources (74% from sulfuric acid and 26% from background).

The $\delta^{34}\text{S}$ of sulfate increased with a decrease in sulfate concentration during the initial stage of the test, attaining a peak of approximately +21‰ in the vicinity of well 729 (Figure 2). This enrichment of the heavier isotope in the residual aqueous sulfate is consistent with bacterial sulfate reduction. Typically, the trend of increasing $\delta^{34}\text{S}$ with decreasing sulfate concentration is observed throughout the entire sulfate reduction process. However, a decrease in $\delta^{34}\text{S}$ values was observed after approximately 70–130 days for the test (Figure 2).

One possible process that could cause the observed decrease of $\delta^{34}\text{S}$ is the release of sulfate from the sediment phase that is less enriched than the extant aqueous sulfate, and the subsequent mixing of the two sulfate sources. The results of the sediment-extraction tests show that the sediment contains a substantial pool of sulfate associated with sulfate-bearing

minerals. The decrease in sulfate concentrations induced by the bacterial sulfate reduction would drive the system farther away from mineral-solution equilibrium, and in effect decrease the apparent saturation index. This would then foment dissolution of sulfate minerals as the system drives to re-establish equilibrium. The results of calculations conducted with the widely used geochemical reaction program PHREEQC indicate that the saturation index for gypsum, for example, would decrease by a factor of approximately two under the site conditions due to the observed decrease in sulfate concentration. Unfortunately, the $\delta^{34}\text{S}$ of the sulfate minerals associated with the sediment is not known. It is feasible that it may be similar to that of the background groundwater (-11.5‰ , measured for groundwater from uncontaminated wells), given that the system is expected to have been in pseudo-equilibrium for many years prior to the impact of the mining activities. In this case, mixing of the sediment-released sulfates with the residual aqueous-phase sulfate would lead to a decrease in the $\delta^{34}\text{S}$ of the mixed aqueous-sulfate pool. The actual mixed value would depend on the relative contributions of the current sources.

The decrease in $\delta^{34}\text{S}$ of sulfate observed in the latter stage of the test could potentially also be explained by the oxidation of hydrogen sulfide back to sulfate. Because the sulfide produced from sulfate reduction is enriched in the lighter isotope compared to the residual sulfate pool, its oxidization back to sulfate and subsequent mixing with the residual sulfate would decrease the aggregate isotopic composition. The potential impact of sulfide re-oxidation may likely be relatively small, however, as discussed above.

Rayleigh Fractionation Modeling

The standard Rayleigh fractionation model was used to simulate the measured isotopic data obtained during the early stage of the test (i.e., wherein $\delta^{34}\text{S}$ increased over time). A best-fit enrichment factor of -5‰ was obtained from the simulations (data not shown). This value is smaller than values reported in the literature, which typically range from -10‰ to -20‰ (e.g., Aravena and Mayer, 2009).

The magnitude or extent of fractionation is expected to be dependent upon a number of factors. For example, the results of a laboratory study showed a much lower average fractionation in systems with non-limiting substrate supply (16‰ to 21‰ at 25°C) compared to systems with limited substrate supply (30‰ to 40‰) (Canfield, 2001). The condition of non-limiting substrate supply was satisfied with the abundant carbon substrate used in this study (1% ethanol solution), which was much greater than the amount required for complete reduction of all nitrate and sulfate in the influenced zone. The type as well as amount of carbon source has a significant influence on the intrinsic enrichment factor (Kaplan and Rittenberg, 1964; Chambers et al., 1975; Habicht and Canfield, 1997). For example, smaller enrichment factors for bacterial sulfate reduction were observed in experiments using ethanol as the carbon substrate compared to other carbon sources (Kemp and Thode, 1968; Canfield, 2001). In a recent laboratory study investigating the role of carbon source on bacterial sulfate reduction of acid mine drainage in lake sediments, enrichment factors as low as -9‰ were observed for experiments with ethanol addition (Fauville et al., 2004).

As discussed above, sulfate-mineral dissolution and oxidation of sulfide to sulfate are both suspected to have been occurring at the site during the test. Both of these processes can impact isotopic composition, and thus influence the apparent enrichment factor. For example, they both can cause $\delta^{34}\text{S}$ of the aqueous sulfate pool to decrease during sulfate reduction, as was observed during the latter stage of the test. Such behavior has been reported in prior studies (e.g., Aravena and Robertson, 1998; Knoller et al., 2006; Wu et al., 2011). Testing the impact of these processes with, for example, a more advanced mathematical model is precluded by the absence of sediment-phase isotopic data.

Impact on Uranium and Long-term Sustainability

The impact of the preliminary biostimulation test conducted using well 765 has been monitored in collaboration with the DOE contractor for four years past the injection. Data for sulfate and oxidation-reduction potential (ORP) are presented in Figure 3. It is observed that reducing conditions were maintained for approximately three years after the single injection, long after the ethanol had dissipated. It is estimated that roughly 10 pore-volume equivalents of groundwater have been displaced through the test zone during this time, based on the natural-gradient flow rate. The monitoring data are insufficient to delineate the reason(s) for this extended sustainability. One hypothesis is that some of the microbial biomass that was produced during the injection period has since died, providing a continued source of electron donor (Borden et al., 2012).

The biostimulation test had a major impact on the disposition of uranium, as shown in Figure 3. Specifically, the aqueous concentration decreased significantly soon after formation of reducing conditions. The low concentrations were maintained throughout the entire time for which sulfate concentrations were low, and rebounded coincident with the increases in sulfate and ORP. It is hypothesized that the apparent sequestration of uranium reflects the impact of microbial reduction, as has been observed in other field tests (Anderson et al., 2003; Istok et al., 2004; Wu et al., 2006; Saunders et al., 2008; Williams et al., 2011). These data suggest that in-situ biosequestration may be a viable option for treating uranium and similar constituents in groundwater systems containing resident sulfate and iron-oxides.

CONCLUSIONS

The impact of electron-donor addition on sulfur dynamics for a system with low levels of metal contaminants was evaluated with a pilot-scale biostimulation test. Significant, long-term decreases in sulfate were observed, and the occurrence of bacterial sulfate reduction was confirmed. A significant portion of the hydrogen sulfide produced from sulfate reduction was possibly sequestered by iron-sulfide precipitation. This indicates that biostimulation is a promising approach for in-situ remediation of sulfate-contaminated groundwater via bacterial sulfate reduction in aquifers that have moderate iron contents. However, such success depends on the aqueous and sediment geochemistry of the subsurface system, as well as other site conditions. Moreover, long-term stability of the precipitates needs additional investigation.

Stable isotope analysis was used to help analyze and interpret the test results. Atypical fractionation was observed wherein the enrichment of $\delta^{34}\text{S}$ in sulfate decreased in the latter stage of the test. Processes such as sulfate-mineral dissolution, sulfide re-oxidation, and iron-sulfide precipitation were hypothesized to have influenced sulfur dynamics, therein affecting observed fractionation. Such behavior has been observed in other field studies (e.g., Bottrell et al., 1995; Knoller et al., 2008; Knoller and Schubert, 2010; Gibson et al., 2011; Wu et al., 2011). The biostimulation test appeared to have caused sequestration of resident uranium, an effect that persisted for approximately three years. The results of this study illustrate the complex biogeochemical behavior that is associated with in-situ biostimulation processes involving bacterial sulfate reduction.

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The impact of electron-donor addition on sulfur dynamics was investigated in a pilot test
Bacterial sulfate reduction was confirmed by monitoring of multiple indicators
Fractionation of $\delta^{34}\text{S}$ for sulfate declined in the latter half of the experiment
The biostimulation test had a major impact on the disposition of uranium in solution

Figure 1.
Schematic map of the site and the test area.

Figure 2.

Sulfate concentrations, hydrogen sulfide concentrations, $\delta^{34}\text{S}$ of sulfate, iron and manganese concentrations for injection well 729 and downgradient wells 730 and 743 after the test.

Figure 3.
Long-term monitoring data for sulfate and uranium.

Table 1

Key water quality parameters for wells 729, 730, and 743

Days	Sulfate (mg/L)	Sulfate (µM/L)	$\delta^{34}\text{S-SO}_4$ (‰)	H_2S (µg/L)	H_2S (µM/L)	DO (mg/L)	NO_3^- (mg/L)	Mn (µg/L)	Fe (µg/L)
Well 729									
0	576	6000	16.2	53	2	2.0	119.2	26	15
41	298	3104	18.0	749	23	0.2	0.5	252	75
69	258	2688	21.1	158	5	0.2	0.2	1000	400
135	212	2208	19.0	30100	941	0.3	4.3	2373	255
205	173	1805	17.4	40000	1250	0.1	ND	1660	445
Well 743									
0	590	6150	16.1	0	0	0.3	123.0	22	3
41	563	5864	16.2	53	2	0.3	122.5	3	60
69	385	4010	17.5	137	4	0.4	121.9	1500	400
135	55	578	16.1	22950	717	0.7	0.6	3724	320
205	5	48	14.0	8000	250	0.2	ND	2229	600
Well 730									
0	571	5950	16.1	16	1	3.6	123.9	5	3
41	577	6005	16.3	50	2	3.5	121.2	12	28
69	446	4646	16.8	17	1	2.0	122.9	800	450
135	51	531	17.0	13950	436	1.1	0.9	3465	770
205	20	212	14.0	21050	658	0.3	ND	2867	434