APPLICATION OF STABLE ISOTOPES AND GEOCHEMICAL ANALYSIS TO
CHARACTERIZE SULFATE, NITRATE, AND TRACE ELEMENT
CONTAMINATION OF GROUNDWATER AND ITS REMEDIATION AT A
FORMER URANIUM MINING SITE

by

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DEDICATION

To life.
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ABSTRACT

Sulfate, nitrate, and certain trace elements are common groundwater contaminants observed at mining sites. Their source, fate, and remediation were investigated at a former uranium mining site. First, groundwater samples collected across the site were analyzed for geochemistry, stable isotopes, and trace elements. Then, two pilot-scale ethanol injection tests were conducted for biostimulation of nitrate and sulfate reduction. Groundwater was monitored in the test area before and after the tests.

The results showed a mixing of two discrete sources of sulfate. Quantification of these two sources using two methods showed that sulfide-mineral oxidation of the mine tailings served as a steady but low-discharge source while sulfuric acid (applied during ore processing in the 1960s) served as a variable, strong source. It appears that sulfuric acid served as a sustained source of sulfate for approximately 40 years. This source may be from accumulation of sulfate salts (formed from sulfuric acid) in the source zone due to the arid climate of the site.

Results showing correspondence of isotopic compositions of ammonium and nitrate confirmed the generation of nitrate via nitrification. Moreover, it was observed that ammonium concentration is closely related to concentrations of uranium and a series of other trace elements including chromium, selenium, vanadium, iron, and manganese. It is hypothesized that ammonium-nitrate transformation processes influence the disposition of the trace elements through mediation of redox potential, pH, and possibly aqueous complexation and solid-phase sorption.
As for the biostimulation, sulfate reduction condition has been maintained for a period of approximately 3 years after a single input. Atypical fractionation behavior of the $\delta^{34}$S in sulfate was hypothesized to be caused by release of sulfate from sulfate minerals associated with the sediments. Elevated hydrogen sulfide concentrations were not observed until approximately four months after the start of the test. This behavior, in concert with the observed changes in aqueous iron and manganese species, suggests that hydrogen sulfide produced from sulfate reduction was precipitated, presumably in the form of iron sulfides, until the exhaustion of readily reducible iron oxides. Hydrogen sulfide produced thereafter appears to have been in part re-oxidized.
CHAPTER 1 INTRODUCTION

1.1 Research Problem

Groundwater contamination at mining sites is a common problem facing the mining industry and environmental researchers. Arizona alone has dozens of registered mining sites that are causing groundwater contamination. Depending on the type of mine and mining techniques, contaminants may include sulfate, nitrate (or ammonium), and a number of trace elements such as uranium, arsenic, chromium, selenium, vanadium, iron, and manganese etc.

Sulfate is a very common groundwater contaminant observed at mining sites. High concentrations of sulfate in drinking water can cause diarrhea in humans, especially infants (EPA, 1999). Water with elevated sulfate has deteriorated taste and order. A secondary MCL of 250 mg/L for sulfate in drinking water is imposed and it is estimated that about 3% of the public drinking water systems in the United States may have sulfate concentrations of 250 mg/L or greater (EPAwebsite, 2013). At mining sites, sulfate is often generated through the oxidation of sulfide minerals (e.g., pyrite) in the mine tailings when exposed to atmospheric oxygen and water, forming so-called acid mine drainage (Johnson and Hallberg, 2005). It may also be from sulfuric acid that is used during ore processing (for certain type of mines). Other potential sources for sulfate that are not related to mining activities include indigenous sulfate mineral dissolution and atmospheric deposition, which are usually of minor importance compared to sulfates generated from mining activities.
Nitrate is another common contaminant of groundwater at mining sites. Elevated nitrate in drinking water can cause serious health effects for infants (blue baby syndrome). Its MCL is regulated as 10 mg/L as nitrogen. The US Environmental Protection Agency (USEPA) has not established a maximum contaminant level (MCL) for ammonia in drinking water. Nitrate can be originated from direct nitrate application during ore processing, oxidation of the reduced form of nitrogen – ammonium - which is also used in ore processing, and sometimes explosives that contain ammonium nitrate (Bosman, 2009). Other potential sources for nitrate that are not related to mining activities are fertilizer application, septic systems, manure application, and erosion of natural deposits.

Mining sites are well-known sources for trace metal contamination. However, most heavy metals are usually constrained in the source zone due to their high adsorption properties. Nevertheless, some trace elements, especially those that form oxyanions in water such as uranium, chromium and arsenic have the potential to travel far in aquifers. Most trace elements are known to be very toxic. Trace element contamination is derived from the release of these elements from ore bodies during ore treatment and mining tailing weathering.

Monument Valley site is a former uranium mining site that has been abandoned for almost 50 years. Extensive sulfate and nitrate (plus ammonium) contamination and minor uranium contamination of groundwater have been observed. Uranium mining at the site occurred from 1943 to 1968. Site history shows that in the leaching processes, sulfuric acid solution was used. After leaching, ammonia and quicklime (calcium oxide) were used to produce a bulk precipitate of concentrated uranium and vanadium. Chemical
solutions and tailings were disposed at two tailing piles without protection. The 
unconfined alluvial aquifer was contaminated with high levels of nitrate and sulfate. The 
contaminant plume emitting from the source zone to the northeast for at least 2 km. 
Concentrations are as high as 170 mg/L (as N) and 1500 mg/L for nitrate and sulfate, 
respectively. Concentrations of ammonium are also as high as 230 mg/L.

Effective control and management of this groundwater contamination requires a 
good identification of the specific source(s). First, identification involves a thorough 
examination of the site historical records, particularly an inventory of chemicals used 
during mining activities, methods of chemical and tailing disposal, and the mineralogy of 
the ores. Second, a site investigation should identify potential sources for groundwater 
contamination. Third, groundwater sampling should be done to characterize the type and 
extent of the contamination. Finally, a combination of hydrogeological, geochemical, and 
isotopic analyses should be used to fully characterize the sources.

A review of site documents shows that two primary potential sources for sulfate 
contamination include the large quantity of sulfuric acid that was used during ore 
processing and sulfate generated from the oxidation of sulfide minerals in the mine 
tailings. Site documents also show that nitrate was in fact used in small amounts during 
ore processing. Thus, the elevated nitrate concentration in groundwater is likely a result 
of oxidation of ammonium, which was used in large quantity during ore processing. 
Previous studies show a slow denitrification process is occurring at the site (Jordan et al., 
2008; Carroll et al., 2009). The first objective of this dissertation research involves the 
characterization of the sources and fate (transformation) of sulfate and nitrate.
Particularly, for sulfate, if sulfuric acid and sulfide-mineral oxidation are truly the two primary sources, their relative contribution, and if sulfate is undergone any natural attenuation (microbially induced sulfate reduction) at the site. For nitrate, specific research question include if the majority of the nitrate is indeed from the oxidation of ammonium (nitrification) and what’s the extent of nitrification compared to denitrification.

Source characterization is only the first step of mining site reclamation management. Remediation and management of contamination is the next step, including that present in the subsurface. Traditional treatment methods for sulfate and nitrate contamination in groundwater include pump and treat, which is inefficient and expensive. Emerging methods include biostimulation, which involves an injection of certain carbon substrate to the subsurface to facilitate anaerobic transformation of these contaminants to non-hazardous chemicals. Biostimulation is well established for treatment of nitrate (denitrification). It transforms nitrate to nitrogen gas, which is non-toxic. In contrast, the product of sulfate reduction is hydrogen sulfide, which is considered a toxin and is easily re-oxidized to sulfate providing an oxic condition. Sulfide is stabilized in the form of metal-sulfide precipitates given the presence of large quantity of heavy metals such as copper and iron which are common in acid mine drainage. Thus, biostimulation is indeed commonly and effectively used in remediation of acid mine drainage (Benner et al., 1997). However, biostimulation for sulfate contamination of groundwater wherein sulfate is the sole contaminant is not well studied. Thus, the effectiveness, the fate of its reduction product hydrogen sulfide, and its impact on aqueous and sedimentary
geochemistry, needs careful evaluation. Two pilot-scale ethanol-injection tests were recently conducted to investigate the efficacy of biostimulation for remediation of nitrate-contaminated groundwater at this site (Borden et al., 2012). The objective of my dissertation research associated with this project (second objective) is to investigate the impact of the biostimulation efforts on the fate of sulfate and associated sulfur dynamics.

Finally, despite the relatively low trace element concentrations within the plume (only uranium exceeds the MCL at two monitoring wells), investigating the disposition of trace elements due to a change in geochemical conditions is valuable and hence is conducted as a minor objective in addition to the two major objectives mentioned above.

### 1.2 Literature Review

**Source Characterization**

Publications on characterization of sulfate sources for groundwater are abundant (Sacks and Tihansky, 1996; Krouse and Mayer, 2000; Einsiedl and Mayer, 2005; Kaown et al., 2009). Publications on sulfate sources at mining sites are also plentiful (Otero and Soler, 2002; Knöller et al., 2004; Dold and Spangenberg, 2005). Methods are quite well-established. Usually, a sound characterization involves the use of a combination of geochemical and isotopic analysis (Krous and Grinenko, 1991; Clark and Fritz, 1997; Krouse and Mayer, 1999). Dual isotope approach has been widely used in source identification of groundwater contamination, on the basis that sulfate (or nitrate) from different sources usually has distinctive isotopic signature on a dual isotope plot (Figure 1) (Yang et al., 1997; Dogramaci et al., 2001; Berner et al., 2002). When mixing of two or
more sources occurs, a spreading of data points between sources on a dual isotope plot can be observed. Should any chemical reactions occur, the data points would deviate from the spreading line (or curve). Take one step further, quantification of two or more sources is possible through the use of end-member mixing analysis (Hooper et al., 1990; Thorburn et al., 1993). Such technique is used to estimate the contribution of each source. No publication has been found on quantifying the contribution of sulfate sources at a mining site.

Figure 1. Typical ranges for δ$_{34}^S$ and δ$_{18}^O$ values of sulfate from various sources (Aravena and Mayer, 2009).

During the transport of sulfate and nitrate contaminants, there can be chemical transformations such as bacterial sulfate reduction, nitrification, or denitrification. These processes can often be observed by apparent concentration change. In addition, change in redox conditions which are manifested by concentration change in aqueous redox
sensitive species (e.g., DO, NO$_3$, Fe, Mn, SO$_4$, H$_2$S) is also good indicator for the confirmation of such processes (Christensen et al., 2000; Christensen et al., 2001). But definitive confirmation is often provided by changes in stable isotopes of the contaminants, because stable isotopes are not affected by physical processes such as dilution and evaporation, but is significantly affected by chemical processes which induce isotopic fractionation between reactant and product. Detailed description of the definition and expression of stable isotopes of sulfur and isotopic fractionation during bacterial sulfate reduction (BSR) is provided in the Appendix A.

Nitrate-containing reagents are generally used in limited quantities, yet extensive nitrate contamination in groundwater has often been observed at uranium mining sites (Landa, 2004). This nitrate appears to have formed via a microbial nitrification process in the subsurface (Ivanova et al., 2000). Conversely, denitrification has also been observed (Longmire and Thomson, 1992). Likewise to sulfate reduction, the occurrence of nitrification or denitrification is determined primarily by the redox conditions, presence of appropriate microbes, and presence of nutrients. The determination of nitrification or denitrification process is also assisted by the use of stable isotopes of nitrate and/or ammonium and the procedure is also very well-established (Kendall and Aravena, 2000). Briefly, during denitrification process the $\delta^{15}$N of nitrate increases with decreasing nitrate concentration while ammonium concentration is not affected. In contrast, during nitrification process the $\delta^{15}$N of ammonium is greater than the $\delta^{15}$N of nitrate because the ammonium with lighter isotopes is preferentially oxidized to nitrate. But as the reaction
proceeds, both ammonium and nitrate will become progressively enriched in $^{15}$N due to Rayleigh distillation.

As for trace elements, it is noticed that ammonium and nitrate have the potential to influence their disposition because ammonium and nitrate transformation reactions affect the redox, the pH, and aqueous complexation and solid-surface adsorption. Preliminary data analysis also shows relationships between ammonium/nitrate concentrations and trace element concentrations for this study site. Literature on the geochemical interaction between nitrogen species (ammonium and nitrate) and trace elements (including uranium) were not found. Management of groundwater contamination at such sites has focused primarily on the remediation of NH$_4$ and/or NO$_3$ (Johnson and Humenick, 1980; Ivanova et al., 2000).

**Remediation**

This dissertation research focuses on the remediation of sulfate in groundwater. The remediation of nitrate is investigated by colleagues (Borden et al., 2012). Detailed literature review regarding the potential use of biostimulation for remediation of sulfate contamination of groundwater and the possible associated sulfur isotopic dynamics is provided in the review article in Appendix A. In short, biostimulation may be a viable approach for the remediation of sulfate contamination of groundwater, depending heavily on the mineralogy of the aquifer sediments and aqueous geochemistry. Sulfur isotopes during bacterial sulfate reduction may be confounded by a number of processes including
sulfate-mineral dissolution, sulfide re-oxidation, and metal sulfide precipitation. Thus, careful interpretation of sulfur isotopes during biostimulation is needed.

1.3 Explanation of dissertation/thesis format

This dissertation consists of two parts, “introduction” and “present study”, and four manuscripts (Appendices A, B, C, and D). The first manuscript (appendix A) was an overview on sulfate reduction in groundwater, published in Environmental Geochemistry and Health, Vol. 34, Issue 4, doi: 10.1007/s10653-011-9423-1, 2012. The other manuscripts (Appendix B, C, D), titled “Quantification of groundwater sulfate sources at a mining site in an arid climate”, “Transport and fate of ammonium and its impact on uranium and other trace elements at a former uranium mill tailing site”, and “The impact of biostimulation on the fate of sulfate and associated sulfur dynamics in groundwater” are to be submitted for peer review. These three manuscripts are associated with sulfate source characterization, nitrate/ammonium transformation and its impact on trace elements, and biostimulation for sulfate remediation, respectively.

I participated in the design and field work of the Monument Valley project, which involved periodic groundwater sampling from selected wells and two ethanol injection tests. I compiled, analyzed, and interpreted the geochemistry, stable isotopes, and trace element data, in order to investigate the sulfate sources, the nitrate/ammonium transformation and its impact on trace elements, and the feasibility of biostimulation for sulfate remediation. I am the primary author for all the appended manuscripts under the guidance of my advisor, Mark L. Brusseau.
CHAPTER 2 PRESENT STUDY

2.1 Summary

The methods, results, and conclusions of this study are presented in the manuscripts appended to this dissertation. The following is a summary of the most important findings in this document.

Sulfate Sources

Two possible sources for sulfate contamination of groundwater were identified based on site history and mineralogy: sulfuric acid released during ore processing and sulfate generated via sulfide-mineral oxidation in the tailing piles. Stable isotope analysis, in combination with geochemical data, was used to characterize the sources and fate of sulfate. The results indicate that bacterial sulfate reduction is insignificant under the extant conditions. The results also indicate the existence of two discrete sources of sulfate. Quantification of these two sources using two methods yielded similar results, given estimates of the original isotopic composition of the two sources. It was shown that sulfide-mineral oxidation served as a steady source with low mass discharge (concentration ~ 117 mg/L) while sulfuric acid served as a highly variable and strong source (44 – 1152 mg/L). Interestingly, it appears that sulfuric acid served as a sustained source of sulfate for approximately 40 years. This may be caused by accumulation of sulfate salts (formed after acid neutralization) in the source zone due to the arid climate of the site. Contrary to the traditional assumption that sulfide-mineral oxidation is the
primary source of sulfate at most mining sites, these sulfate salts are hypothesized to be the primary source for this site.

**Ammonium, Nitrate, and Trace elements**

Geochemical and isotopic results for ammonium and nitrate showed oxic redox conditions and correspondence of isotopic compositions of ammonium and nitrate, which confirmed the natural attenuation of ammonium via nitrification. Moreover, it was observed that ammonium concentration within the plume area is closely related to concentrations of uranium and a series of other trace elements including chromium, selenium, vanadium, iron, and manganese. It is hypothesized that ammonium-nitrate transformation processes influence the disposition of the trace elements through mediation of redox potential, pH, and possibly aqueous complexation and solid-phase sorption. Despite the generally low concentrations of trace elements present in groundwater, their transport and fate may be influenced by remediation of ammonium or nitrate at the site.

**Biostimulation**

The impact of biostimulation on the fate of sulfate and associated sulfur dynamics in groundwater was investigated during ethanol injection tests. Geochemical data and stable-isotope data for sulfate collected before and after the test were analyzed to evaluate the sustainability of sulfate reducing conditions induced by the tests, 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. After the injection, the occurrence of bacterial sulfate
reduction was confirmed by an exponential decrease of sulfate concentration in concert with changes in ORP, redox species, sulfide, alkalinity, and fractionation of δ³⁴S-sulfate. Sulfate reduction condition has been maintained for a period of approximately 3 years. A Rayleigh fractionation model was used successfully to simulate the measured data, with estimated intrinsic enrichment factors ranging from -5 to -9‰. Atypical fractionation behavior of the δ³⁴S in sulfate was hypothesized to be caused by release of sulfate from sulfate minerals associated with the sediments. The Rayleigh simulations incorporated an incrementally increasing degree of sulfate-mineral dissolution as the sulfate reduction process proceeded, and the estimated contribution of dissolution was approximately 10% of the initial sulfate concentration. Elevated hydrogen sulfide concentrations were not observed until approximately four months after the start of the test. This behavior, in concert with the observed changes in aqueous iron and manganese species, suggests that hydrogen sulfide produced from sulfate reduction was precipitated, presumably in the form of iron sulfides, until the exhaustion of readily reducible iron oxides. Hydrogen sulfide produced thereafter appears to have been in part re-oxidized. The results of this study illustrate the complex biogeochemical behavior that is associated with the in-situ biostimulation process for sulfate, and suggest that the success of such a remediation approach depends on the mineralogy of the aquifer, aqueous geochemistry, and other site conditions. A large fraction of sulfate was reduced and sequestered in this study. The long-term stability of the precipitates formed during a biostimulation effort needs further investigation.
2.2 General Conclusions

This dissertation research shows that it is possible to identify the sources of sulfate, nitrate, and trace elements at mining sites using a combination of geochemical and stable isotope approaches. Such source identification contributes to the source control and management of groundwater contamination at mining sites. Specifically, sulfate salt formation from sulfuric acid, accumulation, and subsequent contribution to groundwater sulfate contamination is a new finding that’s contrary to old thoughts that long-term sulfate contamination should be from sulfide mineral oxidation. The study shows that sulfate salts derived from sulfuric acid could serve as a persistent source with even greater strength. This finding has great implication for sulfate source identification for mining sites in the Southwest US where climate allows strong evaporation. Ammonium concentration was found to be closely related to trace element concentrations in groundwater at a mining site for the first time. This finding indicates that trace element concentrations should be closely monitored for mining sites contaminated with ammonium and/or nitrate and are undergoing transformations.

This research is also an initial step towards the remediation of sulfate-contaminated groundwater, which has been a difficult problem facing the mining industry and local communities that are influenced by sulfate contamination of groundwater. This is the first field study in which sulfur dynamics during a biostimulation remediation for sulfate contamination of groundwater were evaluated in detail. The study discovered the impact of sulfide precipitation, sulfate-mineral dissolution, and sulfide re-oxidation on the fate of the total sulfate pool during biostimulation. It also provides a good example in
which the isotopic signature of the total sulfate pool is influenced by a number of factors. It shows that caution should be taken when one wants to interpret change of sulfur stable isotopes during bacterial sulfate reduction. The next step may be a long-term multi-well multi-input field biostimulation test with more detailed aqueous geochemistry, stable isotopes, and especially mineralogy analysis, to investigate the long-term sustainability of the sulfate reducing condition, stability of the sulfide precipitates, and quantification of the precipitates. Future research could also be expanded to other mining sites where sulfate, nitrate, or trace element contamination occurs.
REFERENCES


APPENDIX A: SULFATE REDUCTION IN GROUNDWATER: CHARACTERIZATION AND APPLICATIONS FOR REMEDIATION

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SO4-Overview_rev.doc
Abstract

Sulfate is ubiquitous in groundwater, with both natural and anthropogenic sources. Sulfate reduction reactions play a significant role in mediating redox conditions and biogeochemical processes for subsurface systems. They also serve as the basis for innovative in-situ methods for groundwater remediation. An overview of sulfate reduction in subsurface environments is provided, along with a brief discussion of characterization methods and applications for addressing acid mine drainage. We then focus on two innovative, in-situ methods for remediating sulfate-contaminated groundwater, the use of zero-valent iron (ZVI) and the addition of electron-donor substrates. The advantages and limitations associated with the methods are discussed, with examples of prior applications.

Key words: groundwater pollution; stable isotope analysis; enhanced bioremediation; natural attenuation
1. Introduction

Sulfate occurs extensively in both natural and anthropogenic water systems. Primary natural sources of sulfate include atmospheric deposition, sulfate mineral dissolution, and sulfide mineral oxidation (e.g., Krouse and Mayer, 1999). Anthropogenic sources include coal mines, power plants, phosphate refineries, and metallurgical refineries (e.g., Seller and Canter, 1980). Higher levels of sulfate are common in the western part of the United States (MDH, 2008).

High concentrations of sulfate in ingested water can cause diarrhea in humans, especially infants (EPA, 1999). However, adults generally become accustomed to high sulfate concentrations after a few days (EPA, 1999). Sulfate in drinking water has a secondary maximum contaminant level (SMCL) of 250 mg/L, based on aesthetic effects (i.e., taste and odor) (EPA, 2009). It is estimated that about 3% of the public drinking water systems in the United States may have sulfate concentrations of 250 mg/L or greater (EPAwebsite).

In addition to its classification as a secondary contaminant, there is great interest in the transport and fate of sulfate in subsurface environments in relation to biogeochemical processes affecting natural and contaminated systems. For example, sulfate serves as a primary electron acceptor for anaerobic microbially-mediated transformation processes. Sulfate is reduced during such processes, and thus sulfate reduction is of great significance for many subsurface systems. In addition, sulfate reduction is of particular interest for systems with acidic metal-rich waters, such as mining-impacted sites, because the production of sulfide from sulfate reduction can
effectively precipitate some heavy metals in the form of highly insoluble metal sulfides (e.g., Langmuir, 1997).

In light of growing interest in sulfate as a contaminant and, concomitantly, the feasibility of remediating groundwater contaminated by sulfate, we present an overview of sulfate reduction in subsurface systems. Methods available for characterizing sulfate reduction are discussed. The role of sulfate reduction in addressing acid mine drainage is reviewed. Finally, two innovative, in-situ methods for remediating sulfate-contaminated groundwater, the use of zero-valent iron (ZVI) and the addition of electron-donor substrates, are discussed.

2. Sulfate Reduction in Natural Groundwater Systems

Sulfate is the completely oxidized form of sulfur, and it is the most stable aqueous form of sulfur under aerobic conditions. Sulfur occurs in several oxidation states ranging from $S^{2-}$ to $S^{6+}$, and its geochemical behavior, including transport and fate, is coupled to the aqueous redox conditions (e.g., Hem 1985; Langmuir, 1997). Sulfate is typically the first or second most abundant anion in natural waters (e.g., Hem 1985). Similar to how aqueous carbonate geochemistry is generally considered to exert a primary control over acid-base reactions for natural systems, sulfur geochemistry may be considered to represent a primary control over redox reactions for many environmental systems.

Sulfate reduction occurs extensively in natural groundwater systems. Sulfate is a redox-defining species in the Terminal Electron Accepting Process (TEAP) sequence, with sulfate reduction occurring after iron reduction and before methanogenic conditions.
As such, it is the second most reducing condition in natural groundwater systems. Sulfate reduction involves the consumption of a substantial amount of hydrogen ions and the production of HS\(^{-}\) at circumneutral pH. The general form of the reaction is given as (e.g., Christensen et al., 2000):

\[
\text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{HS}^- + 4\text{H}_2\text{O}
\]  

(1)

Furthermore, dissimilatory sulfate reduction under environmental conditions is conducted by a specialized group of prokaryotic bacteria through chemical reactions in which organic carbon (2) or H\(_2\) (3) is oxidized while sulfate is reduced (e.g., Canfield 2001a,b):

\[
\text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^-
\]  

(2)

\[
2\text{H}^+ + \text{SO}_4^{2-} + 4 \text{H}_2 \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O}
\]  

(3)

Thus, hydrogen ions are consumed and bicarbonate is produced, which leads to increases in pH. These aqueous geochemical signatures along with changes in redox conditions and redox sensitive species may be used as evidence for the occurrence and characterization of sulfate reduction.

Sulfate reduction in the subsurface is almost exclusively mediated by sulfate reducing bacteria (SRB) (e.g., Chambers and Trudinger, 1979; Gibson, 1990; Hao et al., 1996; Canfield, 2001a,b; Berner et al., 2002; and Aravena and Mayer, 2009). These bacteria are anaerobes that gain energy for growth from oxidation of organic matter using sulfate as the terminal electron acceptor (Hao et al., 1996; Barton and Tomei, 1995). While a large number of organic compounds have been shown to support SRB growth (Barton and Tomei, 1995), preferred carbon sources include organic acids (e.g., lactate,
pyruvate, formate, and malate, volatile acids (e.g., acetate), and alcohols (e.g., ethanol, propanol, methanol, and butanol) (Hao et al., 1996). SRB are ubiquitous in the subsurface, and are present (but not likely metabolically active) under aerobic conditions as well as anaerobic conditions (e.g., Gibson, 1990; Bottrell et al., 1991; Fortin et al., 2002; Wargin et al., 2007; Praharaj and Fortin, 2008; Winch et al., 2009). A relatively wide range of genera of SRB has been identified (Hao et al., 1996).

The rate of sulfate reduction in groundwater has been examined in a number of studies. Both zeroth-order and first-order equations have been used to represent field data, depending on prevalent sulfate concentrations. Tabulated zeroth-order reaction rate coefficients for sulfate reduction can be found in several publications (Detmers et al., 2001; Jakobsen and Postma, 1994; McGuire et al., 2002). For example, McGuire et al. (2002) compiled zeroth-order sulfate reduction rate coefficients published in the literature. These values vary from about $2 \times 10^{-6}$ mg/L/day to 3000 mg/L/day. Half-lives associated with first-order sulfate reduction rate coefficients reported for several field studies are compiled in Table 1. Considerable variation is observed among the studies, which may be caused by several factors, including differences in site conditions (e.g., microbial community, redox conditions, aqueous geochemistry, presence of organic contaminants) and measurement methods. The amount of substrate (organic carbon) and nutrients present are often limiting factors for sulfate reduction. For example, larger values are observed for systems with petroleum-hydrocarbon contaminants present compared to uncontaminated aquifers. Finally, it's worth noting that sulfate reduction rate coefficients
have been measured for marine and lake sediments, and are typically orders of magnitude higher than those obtained for aquifers (Jakobsen and Postma, 1994).

3. Characterizing Sulfate Reduction

Several methods or lines of evidence can be used to evaluate the occurrence of sulfate reduction. The baseline approach involves monitoring temporal and spatial changes in sulfate concentrations. However, decreases in sulfate concentrations can be caused by factors other than sulfate reduction, such as hydraulic processes (e.g., dilution) and precipitation of sulfate-bearing minerals (e.g., gypsum). Concurrently, the dissolution of sulfate minerals and the oxidation of sulfide-bearing minerals contribute to increases in sulfate concentrations, which may obscure the occurrence of sulfate reduction. Thus, observation of changes in sulfate concentration is not deterministic evidence for sulfate reduction. Consequently, the use of sulfate concentration data to characterize magnitudes and rate of reduction is influenced by great uncertainty.

As mentioned above, sulfate reduction can significantly impact aqueous geochemical conditions. Thus, changes in concentration of various aqueous-phase constituents are often monitored to evaluate the occurrence of sulfate reduction. For example, reduction of sulfate is often accompanied by the production of \( \text{H}_2\text{S} \), which has a rotten egg odor. This is strong, qualitative evidence for sulfate reduction. However, it is often difficult to quantify the concentration of \( \text{H}_2\text{S} \) present. This is especially true when there are substantial amounts of iron or other metals present, as they can form metal sulfides that may obscure the actual amount of \( \text{H}_2\text{S} \) produced. Sulfate reduction also
affects the speciation and concentrations of metals present, as for example, through formation of metal sulfides and consumption of H⁺ (pH change). In addition, concurrent reduction of Fe(III) and sulfate may occur, which complicates assessment of changes in redox-sensitive species as an indicator of sulfate reduction.

Monitoring concentrations of sulfate and other redox-sensitive species can provide evidence of sulfate reduction. However, as noted, there is often significant uncertainty associated with such evaluations, particularly for quantitative assessments. Stable isotope analysis provides a means by which to improve the assessment of microbially-mediated transformation processes. Specifically, sulfur and oxygen isotopes of dissolved sulfate can be used to confirm the occurrence of sulfate reduction in groundwater.

Stable isotope analysis is based on the isotopic fractionation that occurs during biotransformation of compounds due to differential reaction rates among isotopes of a given element. Specifically, given the dynamics of isotopic fractionation, the parent compound becomes enriched in the heavier isotope and depleted in the lighter isotope as it undergoes biotransformation. The use of isotope fractionation for sulfur has been of interest for some time (e.g., Kaplan and Rittenberg, 1964), and a seminal review of isotopic fractionation for both oxidation and reduction of sulfur was presented by Chambers and Trudinger (1979). A number of studies have investigated sulfur isotope fractionation during sulfate reduction in the subsurface under natural conditions (Strebel et al., 1990; Robertson and Schiff, 1994; Aravena and Robertson, 1998; Krouse and Mayer, 1999; Spence et al., 2001; Berner et al., 2002; Einsiedl and Mayer, 2005; Li et al.,
For sulfate reduction, the residual aqueous sulfate would be enriched in the heavier sulfur and oxygen isotopes (i.e., $^{34}\text{S}$ and $^{18}\text{O}$) versus the lighter isotopes (i.e. $^{32}\text{S}$ and $^{16}\text{O}$) as the reaction proceeds and the concentration of aqueous sulfate decreases.

Sulfur and oxygen isotopes are expressed in delta ($\delta$) notation as $\delta^{34}\text{S}_{\text{SO}_4}$ and $\delta^{18}\text{O}_{\text{SO}_4}$, respectively, which is defined for sulfur as:

$$\delta^{34}\text{S} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \tag{4}$$

where $R_{\text{sample}}$ is the isotopic ratio ($^{34}\text{S}/^{32}\text{S}$) of the number of $^{34}\text{S}$ and $^{32}\text{S}$ atoms measured in a sample and $R_{\text{standard}}$ is the ratio for the reference material, which is a standard recommended by the International Atomic Energy Agency. Values of $\delta^{34}\text{S}_{\text{SO}_4}$ for sulfate that has undergone microbially-mediated reduction range from 20‰-56‰ based on field studies (Berner et al., 2002; Dogramaci et al., 2001; Robertson et al., 1989). Depending on site conditions, the overall sulfur isotope fractionation during bacterial dissimilatory reduction can vary from less than 10‰ to more than 45‰ (Aravena and Mayer, 2009).

Stable isotope analysis has proven to be a useful tool for characterizing sulfate reduction in groundwater for both natural and anthropogenically-affected systems. For example, isotopic analysis has been used to characterize sulfate sources and transformation processes at mining sites (Taylor, 1984; Sracek et al., 2004). However, there are several factors that can complicate and limit the application of stable isotope analysis, and which, in some cases, can result in false positives. For example, different sources of sulfate (e.g., sulfuric acid, atmospheric deposition, mineral dissolution) have different isotopic signatures. Thus, mixing of sulfate from multiple sources could result in
temporal and spatial changes in the composite isotopic signature, which could lead to an incorrect conclusion that sulfate reduction was occurring (a false-positive result). Another constraint to isotope analysis is obtaining robust values for the source signature, which serves as the datum for isotope data analysis. In some cases, the original source may no longer be present at the site. In these cases, the sample with the least enrichment of the heavier isotope (i.e., that subjected to the least amount of transformation) is used as a surrogate. This approach introduces greater uncertainty to the analysis. While stable isotope analysis is a powerful tool, it should be used in combination with other approaches such as geochemical analysis, microbial methods, and mathematical modeling to enhance the robustness of the characterization efforts (e.g., Nijenhuis et al., 2007; Carreón-Diazconti et al., 2009; Carroll et al., 2009).

4. The Role of Sulfate Reduction in Remediation of Acid Mine Drainage

The US Forest Service reported between 27,000 and 39,000 abandoned mines on National Forest System federal lands nationwide (USFS, 2005). Most of these abandoned sites involved metals extraction. A major environmental impact of such mining is the generation of acid mine drainage (or acid rock drainage). Waste rock piles or tailings impoundments are exposed to air and rain that contain oxygen. Sulfide-rich minerals (e.g., pyrite) in the tailings are subject to a series of oxidation reactions that produce sulfate and hydrogen and can lead to the release of heavy metals such as As, Cd, Cu, Ni, Pb, and Zn. The general forms of the acid and sulfate generating reactions are:

$$2\text{FeS}_2(\text{s}) + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 4\text{SO}_4^{2-} + 2\text{Fe}^{2+}$$ (5)
Acid mine drainage has a low pH and typically has elevated concentrations of sulfate and heavy metals. It is estimated that about 20,000 to 50,000 mines are generating acidic drainage on US forest service lands (USDA, 1993). Acid mine drainage may be discharged into surface water and cause significant and persistent contamination. In the western US, between 5,000 and 10,000 miles of streams are impacted by metals mining (USDA, 1993). Infiltration of acid mine drainage can cause significant groundwater contamination.

Johnson and Hallberg (2005) reviewed remediation options for acid mine drainage. Ideally, preventive actions should be taken to control the source to prevent contamination of groundwater. Source control measures include flooding/sealing of underground mines, underwater storage of mine tailings, and land-based storage in sealed waste heaps (Johnson and Hallberg, 2005). These approaches are aimed to prevent the contact of mine tailings with oxygen to minimize the production of acid mine drainage. Common remedial methods for sites with contaminated groundwater can be categorized into abiotic and biological methods, which include both active and passive systems as shown in Figure 1.

Sulfate reduction plays a very important role in the treatment of acid mine drainage because the production of sulfide from sulfate reduction can substantially reduce heavy metal concentrations due to formation of low-solubility metal-sulfide precipitates, and because the coupled oxidation of organic matter in sulfate reduction produces
bicarbonate that serves as a buffer for low pH. It has been shown that SRB can survive in very acid conditions (Church et al., 2007; Koschorreck, 2008). It is worth noting that not only can SRB reduce sulfate, but they can also reduce heavy metals directly. For example, Chang et al. (2001) described SRB that could reduce and immobilize uranium from U(VI) to U(IV) at a uranium mill tailings site. One species, *Desulfotomaculum*, was found to have remarkable tolerance and adaptation to high levels of uranium (Chang et al., 2001).

The use of wetlands is a common approach for treating surface water and groundwater contaminated by acid mine drainage. Anaerobic conditions are maintained due to the abundant natural organic matter, which also serves as substrate for bacteria such as SRB. Sulfate is reduced, forming insoluble metal sulfides and raising pH (Johnson and Hallberg, 2005).

Permeable reactive barriers (PRB) have been increasingly used for passive treatment of groundwater contaminated by acid mine drainage. Benner et al. (1997) described a full-scale PRB installed at the Nickel Rim mine site in Canada for treatment of acid mine drainage. The PRB is composed of 50% organic material, including municipal compost, leaf compost and wood chips, and 50% gravel to maintain high hydraulic conductivity. After passing through the PRB, sulfate concentrations decreased from 2400-4600 mg/L to 200-3600 mg/L; iron concentrations decreased from 250-1300 mg/L to 1.0-40 mg/L; and pH increased from 5.8 to 7.0 (Benner et al., 1997).

In-situ bioprecipitation (ISBP) is an emerging technology that has promise for treating subsurface acid mine drainage contamination. In this technology, electron donors (substrates for bacteria) are injected into wells to stimulate in-situ sulfate reduction and
form a reactive zone for metal precipitation (e.g., Janssen and Temminghoff, 2004; Moon et al., 2009). Substrates that can be injected include stock reagents such as methanol, ethanol, lactate, and hydrogen (Bilek and Wagner, 2009) and less expensive waste products such as manure and sludge (Diels et al., 2005). A successful pilot field application in an aquifer with very unfavorable conditions (high Eh, low pH, low organic matter content, and low sulfate concentrations) illustrates the potential effectiveness of this approach (Janssen and Temminghoff, 2004).

The effectiveness of ISBP depends on many factors, including the type and concentration of amended electron donors, the type and concentration of contaminants, background sulfate concentration, pH, redox potential, and soil type (Diels et al., 2005). The selection of appropriate electron donors is generally the most important factor, as it influences the rates of reaction and the type and stability of the metal precipitates (Diels et al., 2005). Potential drawbacks to ISBP include non-persistence of precipitates after termination of substrate supply, potential clogging of pore spaces, and long lag time (Bilek, 2006; Vanbroekhoven et al., 2008). The long-term stability of the metal-sulfide precipitates generated is generally the primary issue for this method. While the results of some studies suggest that some metal precipitates do not undergo significant leaching (Bilek, 2006; Schols et al., 2008; Vanbroekhoven et al., 2009), the results of others have shown they are not always stable under various conditions (Beyenal and Lewandowski, 2004; Prommer et al., 2007).
5. Remediation of Sulfate-Contaminated Groundwater

Pump and treat is the standard method used for remediation of groundwater for which sulfate is the primary contaminant. This approach is effective at controlling the contaminant plume, but is generally cost and time intensive. Electrokinetic methods are another possible in-situ alternative for remediation (e.g., Runnells and Wahli, 1993). However, their use would typically be restricted to very small, shallow sites with relatively high concentrations. Interest is growing in the use of methods that are based on microbially-mediated processes as an alternative or adjunct to pump and treat. Two innovative, in-situ methods under current investigation involve the use of zero-valent iron (ZVI) and the addition of electron-donor substrates.

Zero valent iron has been used successfully for in-situ treatment of organic compounds such as chlorinated solvents, as well as for a range of inorganics such as arsenic (Kanel et al., 2005; Lackovic et al., 2000), nitrate (Della Rocca et al., 2007; Yang et al., 2008), heavy metals (e.g., nickel, mercury, silver, and cadmium), and radionuclides (e.g., UO$_2^{2+}$) (Cantrell et al., 1995; Li et al., 2006; Ponder et al., 2000; Zhang, 2003). The results of several laboratory studies have indicated that significant decreases in sulfate concentrations can be achieved with treatment by ZVI (Bilek, 2006; Bilek and Wagner, 2009; Burghardt et al., 2007; Diels et al., 2005; Janssen and Temminghoff, 2004). Zero-valent iron can react directly with sulfate via abiotic reaction (Equation 7). More significantly, ZVI reacts with water to produce H$_2$ and ferrous iron (Equation 8), after which sulfate is reduced by H$_2$ to sulfide via microbially-mediated reactions and forms iron sulfide precipitates (Equation 9):
\begin{align*}
\text{Fe}^0_{(S)} + \frac{1}{4}\text{SO}_4^{2-} + 2\text{H}^+ & \rightarrow \frac{1}{4}\text{FeS}^{(S)} + \frac{3}{4}\text{Fe}^{2+} + 10\text{H}_2\text{O} \quad (7) \\
\text{Fe}^0_{(S)} + 2\text{H}_2\text{O} & \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^- \quad (8) \\
2\text{Fe}^{2+} + \text{SO}_4^{2-} + 4\text{H}_2 & \rightarrow \text{FeS}^{(S)} + 4\text{H}_2\text{O} \quad (9)
\end{align*}

The results of laboratory studies have shown that minimal decreases in sulfate typically occur in the presence of sterile ZVI, indicating very low rates of abiotic sulfate reduction (Burghardt et al., 2007; Karri et al., 2005). Conversely, much higher rates of reduction are observed for systems with SRB present (Karri et al., 2005), which indicates the prime importance of microbially-mediated reduction for sulfate.

There are two primary methods by which ZVI may be employed. The common approach involves a permeable reactive barrier system, wherein the ZVI is placed within a trench to control for example the leading edge of a contaminant plume. To date, PRB applications for inorganics have been designed to target various metals, and have not been used for cases wherein sulfate is the sole contaminant. However, in some cases, sulfate was present as a co-contaminant, and significant concentration decreases were observed along with induced precipitation of metal sulfides (e.g. Johnson et al., 2008; Ludwig et al., 2009; Wilkin et al., 2005). Successful use of ZVI for sulfate treatment would possibly entail supplementation with an amendment to enhance activity of SRB populations.

The second approach for use of ZVI involves injection of colloidal forms of ZVI to target specific zones of contamination. Several field studies have been conducted
illustrating the injection of colloidal ZVI for remediation of chlorinated-solvent contamination (Bennett et al., 2010; Elliott and Zhang, 2001; Elsner et al., 2010; Henn and Waddill, 2006; Quinn et al., 2005; Wei et al., 2010; Zhang, 2003). To the best of our knowledge, field studies of direct injection of colloidal ZVI for sulfate contamination have not been reported.

The advantages of colloidal ZVI injection compared to PRB systems are greater flexibility in targeting the source and high reactivity due to extremely high specific surface area of the colloidal ZVI (typically up to 30 times greater than granular iron used for PRBs) (Zhang, 2003). However, the drawbacks include relatively short longevity of the colloidal ZVI (weeks compared to years with PRB) and potential limited transport due to aggregation and strong attachment (He and Zhao, 2007; He et al., 2010; Saleh et al., 2005; Schrick et al., 2004; Zhang, 2003). Highly dispersed nanoscale ZVI particles stabilized with carboxymethyl cellulose (CMC) have been used to improve delivery (He and Zhao, 2005; He and Zhao, 2007; He et al., 2007). However, the results of field studies showed that transport distances were still limited for injected particles, with estimated maximum travel distances in the range of 1-2 meters (Bennett et al., 2010; He et al., 2010). Given these disadvantages, it appears that ZVI injection may be best suited for small, localized contaminant sources.

There is great interest in the use of electron-donor substrates to promote microbially-mediated reduction for the remediation of groundwater contaminated by a variety of inorganic elements. For example, this approach has been applied to systems with uranium-contaminated groundwater (Istok et al., 2004; Wu et al., 2006; Wu et al.,
Sulfate was a co-contaminant in several of these studies, and significant decreases in sulfate concentrations were typically observed after substrate addition. Based on these results, it is likely that addition of electron-donor substrates would serve as a potential method for sulfate remediation for systems wherein it is the principal contaminant.

Several laboratory studies have been conducted to evaluate the impact of electron-donor addition on sulfate reduction for various applications, including acid mine drainage (e.g., El Bayoumy et al., 1999; Glombitza, 2001; Johnson and Hallberg, 2002; Gibert et al., 2002), recovery of industrial wastewater (e.g., García-Saucedo et al., 2008; Eljamal et al., 2009; Meulepas et al., 2010), remediation of contaminated lakes and sediments (e.g. Smith and Klug, 1981; Lovley et al., 1995), and to investigate isotopic fractionation (e.g. Bolliger et al., 2001; Kleikemper et al., 2004). Results from these studies indicate that electron donors are essential for the treatment of sulfate-containing water and sediments by biologically mediated sulfate reduction. It is also clear that different electron donors can result in different magnitudes and rates of reaction. Liamleam and Annachhatre (2007) summarized sulfate reduction data including reduction rates, microbial biomass yield, and free energies of reactions by electron donor.

A primary concern for the use of the electron-donor approach for sulfate is the fate of sulfide produced from sulfate reduction. For the wetlands and ISBP technologies noted above for treatment of acid mine drainage, the sulfide is primarily sequestered through formation of metal-sulfide precipitates because of the abundance of metals
generally associated with these systems. For the situation wherein sulfate is the primary contaminant, the fate of the sulfides will depend upon site conditions. If one or more metals (e.g., Fe, Mn) are abundant, the sulfide may form precipitates and be sequestered. The long-term stability of these precipitates would be of concern, as discussed above for the ISBP method. For sites with low abundance of metals, sulfate will to a great extent be converted to hydrogen sulfide. For circumneutral pH conditions, HS⁻ will predominate, and it will be subject to aqueous-phase advective-dispersive transport. For lower-pH conditions, hydrogen sulfide gas may form, which can transport via gas-phase processes. Hydrogen sulfide is quite labile, and subject to re-oxidation to sulfate when it encounters groundwater with higher redox potential.

A pilot test using electron-donor addition for remediation of nitrate and sulfate contaminated groundwater was recently conducted at a former uranium mining site in Arizona. Ethanol was used as the substrate, and the test was conducted using the single-well injection method (Borden, 2010). The results showed that the concentration of nitrate and dissolved oxygen decreased (Figure 2), while the concentration of nitrous oxide (a product of denitrification) increased. Continued monitoring after completion of the pilot test has shown that nitrate concentrations surrounding the injection well have remained at levels three orders of magnitude lower than the initial value (~120 mg/L as N), indicating that the impact of the pilot test has been sustained for several months. Concentrations of sulfate were observed to decrease significantly after the ethanol injection test (see Figure 3), with concentrations of approximately 600 mg/L observed before the test decreasing to approximately 10 mg/L within one year after the test.
Concomitantly, concentrations of hydrogen sulfide were observed to increase. These results suggest that introduction of the electron donor resulted in significant decreases in concentrations of both nitrate and sulfate via microbially-mediated reduction. The formation and fate of reaction products such as hydrogen sulfide, which will impact the long-term viability of this approach, need to be evaluated.

7. Summary

Sulfate is ubiquitous in groundwater, with both natural and anthropogenic sources. It is often used to help characterize hydrologic and biogeochemical interactions in subsurface environments. The standard approach for remediating groundwater contaminated by sulfate, pump and treat, is relatively expensive and time intensive. Innovative remediation methods include the use of zero valent iron or electron-donor substrates to promote in-situ sulfate reduction, primarily via microbially-mediated processes.

Pilot tests employing ZVI for other contaminants have been conducted successfully, and sulfate, present as a co-contaminant, has been observed to undergo reduction. These results indicate that the use of ZVI may be a viable approach for remediation of sulfate-contaminated groundwater. The results of a pilot-scale electron-donor test illustrate that the injection of a substrate to enhance biotransformation can effectively reduce sulfate concentrations. Enhanced reduction thus appears to also be a viable method for in-situ remediation of sulfate-contaminated groundwater. However,
there is a need to evaluate potential constraints associated with these approaches, and to identify site conditions that would support successful implementation.

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Table 1. Summary of first-order sulfate reduction half-lives from field studies.

<table>
<thead>
<tr>
<th>Location</th>
<th>Method</th>
<th>Sulfate Conc. (mg/L)</th>
<th>Half-lives</th>
<th>Contamination</th>
<th>Citation</th>
</tr>
</thead>
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<tr>
<td>Contaminated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wurtsmith AFB, MI</td>
<td>Single-well push pull (BTC)</td>
<td>10</td>
<td>0.1-0.2 days</td>
<td>Petroleum and chlorinated solvents</td>
<td>(McGuire et al., 2002)</td>
</tr>
<tr>
<td>Studen, Switzerland</td>
<td>Single-well push pull (BTC)</td>
<td>96</td>
<td>5-16 days</td>
<td>Petroleum hydrocarbons</td>
<td>(Schroth et al., 2001)</td>
</tr>
<tr>
<td>Seal Beach, California</td>
<td>Tracer Test (BTC)</td>
<td>80</td>
<td>7 days</td>
<td>Fuel hydrocarbons</td>
<td>(Cunningham et al., 2000)</td>
</tr>
<tr>
<td>Hanahan, South Carolina</td>
<td>Flowpath (Conc. Vs Distance)</td>
<td>0-14</td>
<td>9-35 days</td>
<td>BTEX</td>
<td>(Chapelle et al., 1996)</td>
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<tr>
<td>Fuhrberger, Germany</td>
<td>Depth profile (GW age vs conc.)</td>
<td>105-175</td>
<td>30-40 years</td>
<td>None</td>
<td>(Strebel et al., 1990)</td>
</tr>
<tr>
<td>Sturgeon Falls, Canada</td>
<td>Depth profile (GW age vs conc.)</td>
<td>6-27</td>
<td>1 year</td>
<td>None</td>
<td>(Robertson and Schiff, 1994)</td>
</tr>
<tr>
<td>Uncontaminated</td>
<td></td>
<td></td>
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Figure Captions

Figure 1. Remediation options for acid mine drainage; figure reproduced from Johnson and Hallberg (2005).

Figure 2. Concentration of nitrate and dissolved oxygen for a pilot test of ethanol amendment injection conducted at Monument Valley, AZ.

Figure 3. Sulfate concentrations measured for the pilot test conducted at Monument Valley, AZ. The zero point for elapsed time represents the start of the pilot test.
Fig. 1

REMEDICATION

- Abiotic
  - "Active" systems: aeration and lime addition
  - "Passive" systems: e.g. anoxic limestone drains

- Biological
  - "Active" system
    - Off-line sulfidogenic bioreactors
  - "Passive" systems
    - Aerobic wetlands
    - Compost reactors/wetlands
    - Permeable reactive barriers
    - Packed bed iron-oxidation bioreactors
Fig. 2
Fig. 3
APPENDIX B: QUANTIFICATION OF GROUNDWATER SULFATE SOURCES AT A MINING SITE IN AN ARID CLIMATE

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Abstract

Sulfate contamination of groundwater is commonly observed at many mining sites, especially coal mines and sulfide-rich metal mines. The Monument Valley DOE site, a former uranium mining site located in the Southwest U.S., is marked with high groundwater sulfate concentrations. Two possible sources were identified based on site history and mineralogy: sulfuric acid released during ore processing and sulfate generated via sulfide-mineral oxidation in the tailing piles. Stable isotope analysis, in combination with geochemical data, was used to characterize the sources and fate of sulfate. The results indicate that bacterial sulfate reduction is insignificant under the extant conditions. The results also indicate the existence of two discrete sources of sulfate. Quantification of these two sources using two methods yielded similar results, given estimates of the original isotopic composition of the two sources. It was shown that sulfide-mineral oxidation served as a steady source with low mass discharge (concentration ~ 117 mg/L) while sulfuric acid served as a highly variable and strong source (44 – 1152 mg/L). Interestingly, it appears that sulfuric acid served as a sustained source of sulfate for approximately 40 years. This may be caused by accumulation of sulfate salts (formed after acid neutralization) in the source zone due to the arid climate of the site. Contrary to the traditional assumption that sulfide-mineral oxidation is the primary source of sulfate at most mining sites, these sulfate salts are hypothesized to be the primary source for this site.
Keywords
sulfate, groundwater, contamination, stable isotopes, sulfide-mineral oxidation, mining, mill tailing

Introduction
Sulfate released from mine tailings has become a recent environmental concern. Sulfate contamination of groundwater or surface waters is associated with aesthetic problems (bad odor), and may cause diarrhea (e.g., EPA, 1999). It is also associated with other issues such as corrosion of plumbing systems (e.g., Djuric et al., 1996) and enhanced methylation of mercury leading to accumulation of mercury in fish when released into surface water (e.g., Jeremiason et al., 2006).

Mines, especially coal mines and metal mines that involve large quantities of sulfide minerals, often serve as sources for sulfate emission into groundwater (Johnson and Hallberg, 2005). Abandoned mines are often sources of sulfate contamination due to a lack of control and management procedures in the past. It is estimated that there are at least 161,000 abandoned hardrock mine sites in the 12 western states and Alaska in the U.S. (GAO, 2011). The common source of sulfate at mining sites is the oxidation of sulfide minerals (e.g., pyrite) in mine tailings and waste rocks that are exposed to air. At some mining sites, sulfuric acid was used during ore processing (extraction of metals). Although the sulfuric acid is subsequently neutralized by regents (e.g., lime and ammonia), the sulfate ion is not eliminated and thus constitutes a direct source for groundwater contamination. Other sources of sulfate in groundwater may include
indigenous sulfate minerals and atmospheric deposition. The latter is generally expected to be negligible (concentration in precipitation is ~1 mg/L) (NADP, 2011) compared to the high concentrations (hundreds of mg/L) typically observed at mining sites.

It is critical to understand the sources of sulfate released from mining sites both qualitatively and quantitatively for better pollution prevention and for improved management of existing contamination. Such understanding may be supported by the use of geochemical and possibly isotopic approaches. Stable sulfate isotopes have long been used in characterizing and delineating sulfate sources (e.g., Krouse and Grinenko, 1991; Krouse and Mayer, 1999). However, while the procedure is well established, there are often factors that complicate its use such as mixing (dilution), sulfate-mineral dissolution, and microbial sulfate reduction that also affect the isotopic composition of the total aqueous sulfate pool. Thus, accurate assessment of sulfate sources requires a thorough understanding of the geochemical (including redox) and hydrological aspects of the site.

Extensive sulfate groundwater contamination is present at the Monument Valley DOE site, a former uranium mining site in southwest U.S. Two potential sources of sulfate were identified according to the site history and mineralogy: the first from the sulfuric acid applied during ore leaching and the other from sulfide-mineral oxidation in the mine tailings. The objective of this study is to evaluate the relative contribution of these two sources and to identify possible processes (e.g., mixing and bacterial sulfate reduction) that affect the fate of the sulfate. This is accomplished using a combination of geochemical, hydrogeological, and isotopic analyses.
Site Conditions

The Monument Valley site is located in northeastern Arizona, 24 km south of Mexican Hat, UT. The site is approximately 1,460 m above sea level, and the climate is arid. The site receives approximately 160 mm of precipitation annually (DOE, 2005). Most precipitation usually occurs during July through August and December through February (DOE, 2005). Temperature is high in the summer (32 - 38°C), and can be as low as the freezing point in the winter.

Uranium mining at the site occurred from 1943 to 1968. During that time the site was one of the largest producers of uranium in the state of Arizona (with a production of 767,166 tons of uranium and vanadium ore). From 1955 to 1964, ore at the site was processed by mechanical milling with an upgrader, which crushed the ore and separated by grain size. Finer-grained material was shipped to other mills for chemical processing while coarser-grained material stored on site in the “old tailing pile”. The only chemical used during this period of time was minor amounts of flocculent. From 1964 to 1968, batch leaching and heap leaching were used to process an estimated 1.1 million tons of tailings and low-grade ore at the site. In both leaching processes, sulfuric acid solution was used, which is believed to be a primary source of sulfate contamination of groundwater. After leaching, ammonia and quicklime (calcium oxide) were used to produce a bulk precipitate of concentrated uranium and vanadium. Chemical solutions used in ore processing are believed to have been discharged to the “new tailings pile.” The new tailings pile contained both sandy tailings and processing solutions. An evaporation pond was on the east side of the new tailings pile. The purpose of the
evaporation pond is unknown, but it may have been used to retain seepage from the new tailings pile. Surface remediation at the site took place from 1992 through 1994 and resulted in the removal of these source materials and other site-related contamination.

The mineralogy of the ore body at the site consists of uranium and vanadium minerals, which occur with copper minerals and cementing minerals consisting of quartz and calcite (Lewis and Trimble, 1959). Sulfide minerals such as copper sulfides, chalcocite, chalcosulfide, and bornite are common in the ore (Lewis and Trimble, 1959). Exposure of these sulfide minerals to the atmosphere and subsequent oxidation due to the disposal of mine tailings containing these minerals can produce sulfate. This is considered another primary source of sulfate at this site.

The shallow alluvial aquifer is comprised of well-sorted fine to medium sand deposits interspersed with finer silt and clay sediments. Generally, the alluvial deposits range from 1 to 35 meters in thickness, with the greatest depths found in the center of the valley. Depth to groundwater is approximately 11 meters. The screened intervals for most monitoring wells span approximately 15 to 25 m below ground surface. Bedrock is exposed at the western and southern area of the source zone, where recharge occurs. This results in a general northeastward hydraulic gradient. The mean hydraulic gradient is approximately 0.01. Hydraulic conductivities range from 0.1 to 5.6 m/day for the entire alluvial aquifer (DOE, 1999). The mean groundwater pore water velocity can be calculated to be 44 m/yr with a known porosity of 0.25 and an assumed hydraulic conductivity of 3 m/d. Pore water velocity directly measured from a tracer test on a scale
of 10m is 30 m/yr. Nitrate, sulfate and uranium are the primary groundwater contaminants.

**Materials and Methods**

**Sample Collection and Analysis**

Groundwater water samples were collected from 14 selected wells across the entire plume. In addition, several wells upgradient of the source zone were sampled for background geochemistry. Samples were collected using dedicated bladder pumps and a QED Micropurge controller. Sampling began once field parameters (pH and dissolved oxygen) stabilized, which typically occurred after the removal of a few borehole-equivalent volumes of groundwater. Samples were first collected for measurement of basic field parameters (dissolved oxygen, pH, oxidation-reduction potential, and temperature), then for cations/anions, and finally for sulfate isotopes. Samples for cations and anions were filtered (0.45 μm) and collected in 250-mL HDPE bottles, separately. Cation analysis samples were preserved with 1 mL of 1:1 hydrochloric acid (HCl). Both sample sets were stored on ice at 4°C. The isotope samples were filtered (0.45 μm) and collected in separate HDPE bottles of various sizes depending on the concentration. Hydrochloric acid was added to decrease pH to < 2 for preservation. All samples were stored on ice in the field then frozen upon returning to the lab.

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 (e.g., nitrate and sulfate) were analyzed using a Dionex DX-1000 following EPA
standard method 300.0. NH$_4^+$ analysis was conducted using a Hach colorimetric kit and analyzed on a Hitachi U-2000 Spectrophotometer. MDLs are 0.1 mg/L for NO$_3^-$ and NH$_4^+$ and 0.5 mg/L for Cl$^-$ and SO$_4^{2-}$.

The sulfur isotopes of sulfate were measured for SO$_2$ gas with a continuous-flow gas-ratio mass spectrometer (ThermoQuest Finnigan Delta Plus XL) at the Environmental Isotope Laboratory of the University of Arizona. Calibration was linear in the range -10 to +30 per mil. Precision was estimated to be ± 0.15 or better (1 standard deviation), based on repeated internal standards. Oxygen isotopes of sulfate were measured on CO gas in a continuous-flow gas-ratio mass spectrometer (Thermo Electron Delta V). Samples were combusted with excess C at 1350 °C using a thermal combustion elemental analyzer (ThermoQuest) coupled to the mass spectrometer. Precision is estimated to be ± 0.4 per mil or better (1 st.dev.), based on repeated internal standards. Standardization is based on international standard OGS-1, and results were reported in delta notation (δ) in per mil units (‰).

**Data Analysis**

The dual-isotope approach (δ$^{18}$O vs δ$^{34}$S) was used to identify the sulfate sources, based on the concept that sulfate derived from different sources usually has distinct δ$^{18}$O and δ$^{34}$S signatures. This approach provides improved differentiation of potential sources of sulfate compared to using sulfur isotopes alone. It also provides, under certain conditions, patterns that can be used to indicate processes (e.g., mixing or bacterial sulfate reduction) that are affecting the sulfate sources. Moreover, an end-member mixing
analysis can be used to quantify the sources if the dual-isotope plot shows a mixing trend of two or more sources (e.g., Hooper et al., 1990). In the case of two end-members, knowing the original isotopic composition of the two sources (δ₁ and δ₂) and the mixture (δ_{mix}), and the total concentration of the mixture (C_T) allows the calculation of the concentration of each source (C₁ and C₂). The difficulty or uncertainty often lies in determining the original isotopic composition of the two sources.

A technique of plotting the isotopic composition relative to the inverse of concentration is also used to assess the mixing process. The basis for this method is that, due to mass conservation, $C_T \delta_{mix} = C_1 \delta_1 + C_2 \delta_2$, and $C_T = C_1 + C_2$, where δ and C designate isotopic composition and concentration, respectively, and subscripts 1, 2, mix, and T designate source 1, source 2, the mixture, and total concentration, respectively. Rearranging these two equations in terms of δ_{mix}:

$$\delta_{mix} = \frac{C_1}{C_T}(\delta_1 - \delta_2) + \delta_2.$$ Therefore, if a linear relationship is yielded by plotting the isotopic composition of the mixture against the inverse of the total concentration, the slope equals $C_1(\delta_1 - \delta_2)$ and the y-intercept equals the isotopic composition of the second source. Note that this method is valid only when C₁ (the concentration of the first source) is constant. Thus, it is often used when a source with a constant background concentration is mixed with another source with a highly variable concentration (e.g., Krouse and Mayer, 1999; Miller and Tans, 2003).

The concentration of the constituent of interest can be normalized by a presumably conservative constituent to attempt to eliminate the effects of dilution or evapotranspiration (e.g., Krouse and Mayer, 1999). In such a case, the equation above
becomes $\delta_{mix} = \frac{1}{(\frac{c_T}{c'})} \left( \frac{c_1}{c'} \right) (\delta_1 - \delta_2) + \delta_2$, where $C'$ is the concentration of the conservative tracer. The slope equals $\frac{c_1}{c'} (\delta_1 - \delta_2)$ . Quantification of each source concentration is also possible using this method. $C_1$ is calculated using equation slope = $\frac{c_1}{c'} (\delta_1 - \delta_2)$ by obtaining the slope and knowing $\delta_1$, $\delta_2$, and $C'$ (if $C'$ is used). $C_2$ simply equals $C_T - C_1$. The results obtained from this mass-conservation mixing analysis were compared to those obtained with the dual-isotope method.

**Results and Discussion**

**Geochemistry**

The groundwater geochemistry is summarized in Table 1. The background groundwater geochemistry, inferred from upgradient uncontaminated wells, shows a transition from calcium-magnesium-carbonate type to sodium-sulfate type. The sodium-sulfate composition is suspected to be a result of local evapotranspiration (DOE 2005). Generally, the contaminated groundwater shows much higher concentrations of calcium (105.0 ± 39.1 mg/L), magnesium (85.5 ± 36.4 mg/L), sulfate (574.7 ± 388.4 mg/L), nitrate (101.9 ± 67.6 mg/L) and ammonium (74.7 ± 81.7 mg/L) than the background groundwater (Ca = 21.2 ± 7.0, Mg = 28.8 ± 12.2, SO4 = 70.9 ± 41.2, NO3 = 0.4 ± 0.3, NH4 = 0.0 ± 0.1, respectively). Similar water chemistry (sulfate, calcium, and bicarbonate rich) has been observed at other mining sites (e.g., Toran, 1987; Armienta et al., 1997).

The Ca and Mg were likely introduced by the quicklime that was applied at the end of ore processing for neutralization. The sulfate is presumed to originate from direct
input during ore processing (sulfuric acid) or sulfide oxidation in the tailing pile as mentioned above. The ammonium is from ammonia application during ore processing, and the nitrate is most likely from nitrification of ammonium (Carroll et al., 2009). The contaminated groundwater has a lower pH (7.4 ± 0.2) than the background groundwater (8.0 ± 0.3). This is likely a result of a loss of buffering from carbonate minerals (calcite and dolomite) present in the sediments due to their dissolution associated with acid use during ore process and/or acid release during subsequent sulfide-mineral oxidation.

The mean sulfate to chloride ratio is 29.3 for contaminated groundwater compared to a value of 3.9 for background groundwater (Table 1). However, the chloride concentration of contaminated groundwater (mean = 19.6 mg/L) is comparable to the background chloride concentration (mean = 18.4 mg/L). This indicates that the input of sulfate is accompanied by negligible chloride. Chloride input from ore processing or subsequent weathering is expected to be negligible because no chloride species were used in the ore processing, and the minerals associated with the mine tailings lack chloride. With minimal input of chloride associated with mining activities at the site, it is reasonable to assume that chloride can serve as a conservative tracer in this study. The role of chloride during the transport of sulfate will be further discussed in the following sections.

The redox condition of the background groundwater is oxidizing (ORP ~ 299 mV) (DOE, 1999). The contaminated groundwater is generally under oxidizing conditions as well, reflected in DO values ranging from 0.9 to 12.7 mg/L. Nitrate concentrations (10.9 – 223.8 mg/L as N) are elevated in all contaminated wells. Iron and manganese
concentrations are generally below 100 μg/L and vary significantly for both contaminated and background groundwater. No H₂S was detected in a subset of wells located in the middle (near well 765) of the plume. All of the above redox-indicating species (DO, NO₃, Fe, Mn, and H₂S) are consistent with oxic conditions. Such redox conditions are not suitable for bacterial sulfate reduction.

The sulfate plume emanating from the source zone extends for 2 km to the northeast. Concentrations including those in background wells range from 17 – 1500 mg/L (Figure 1). Within the plume, concentrations are generally > 100 mg/L. Unlike many contaminated sites where the highest concentrations occur in the source zone, concentrations within the source zone are low (generally < 100 mg/L), which is likely due to the remediation of the surface area of the source zone during 1992 to 1994. The highest sulfate concentrations at this site are observed at two hot spots, 450m (well 771) and 1400m (well 762) downgradient of the source zone, respectively.

Following the surface remediation efforts, the source of extensive sulfate input was presumably terminated. This termination of the source would result in development of a zone of higher concentration (hot spot) that would migrate downgradient at the effective groundwater velocity. Given a groundwater pore velocity of 30 m/yr (measured by tracer test) and a travel time of 14 years (1994 – 2008), it is estimated that the hot spot should currently be located approximately 420 m from the source area. This coincides well with the location of the closer observed hot spot (450 m).

The more distant hot spot is probably caused by the sulfuric acid application during 1964 to 1968. Given the approximate travel time of 42 years (1966 – 2008) and
groundwater velocity of 30 m/yr, the travel distance is estimated to be 1260 m, which is in general agreement with the distance of the distal hot spot (1400 m) from the source area. Therefore, conceptually, it appears that the distal hot spot is caused by direct input of sulfate from sulfuric acid used during the mill operation, while the remainder of the plume is caused by release of sulfate from sulfide-mineral oxidation in the tailing pile prior to remediation of the source area.

**Stable Sulfate Isotopes**

The sulfur and oxygen isotope composition of sulfate exhibited a strongly linear relationship with a slope approximately equal to 1:4 on a dual-isotope ($\delta^{18}$O vs $\delta^{34}$S) plot (Figure 2). Figure 2 also shows the ranges of isotopic composition of sulfate from different sources. The top end of the linear regression line is in the region associated with evaporites, while the bottom end can be extrapolated into the region associated with sulfide-mineral oxidation (Figure 2).

A linear relationship between $\delta^{18}$O and $\delta^{34}$S is consistent with mixing of two sulfate sources with differing isotopic composition. However, bacterial sulfate reduction also results in simultaneous enrichment of $^{18}$O and $^{34}$S. In fact, results reported in the literature suggest a slope of 1:4 for $\delta^{18}$O vs $\delta^{34}$S during bacterial sulfate reduction (e.g., Mizutani and Rafter, 1973), which coincides with the slope in Figure 2. To delineate the cause of the linear relationship between $\delta^{18}$O and $\delta^{34}$S, $\delta^{34}$S is plotted relative to sulfate concentration (Figure 3). A general positive trend is observed, which is inconsistent with bacterial sulfate reduction because $\delta^{34}$S increases with decreasing sulfate concentration.
during the biotransformation process (the heavier isotopes accumulate in the residual sulfate). This result combined with the redox conditions present at the site indicate that the linear relationship is not caused by bacterial sulfate reduction. Concomitantly, this suggests that a two-source mixing process is responsible.

If the observed isotopic behavior is a result of mixing, the two sources should be sulfuric acid and sulfide-mineral oxidation as identified above. The original isotopic composition of the sulfate generated from sulfide-mineral oxidation should be associated with the region of sulfide-mineral oxidation in Figure 2 (bottom end of the regression line). Therefore, the other end of the regression line (within the range of evaporites in Figure 2) must be associated with the isotopic composition of the sulfuric acid. The isotopic composition of industrial sulfuric acid depends on the isotopic composition of the raw materials. Therefore, it is possible to have an isotopic composition in the range of evaporites if the sulfuric acid used at the site was produced from evaporites. Despite the fact that presently most sulfuric acids have a $\delta^{34}S$ value close to 0 due to their production from oxidation of sulfur that has $\delta^{34}S$ value close to 0, values of $\delta^{34}S$ as high as 18‰ have been reported for sulfuric acid (Hesslein et al., 1988).

To further assess the mixing process, the sulfur isotope composition of sulfate is plotted relative to the inverse of sulfate concentration. The results show a linear negative relationship (slope = -1727.4, intercept = 17.9, $R^2 = 0.663$) (Figure 4, top). This inverse relationship is significantly improved when sulfate concentration is normalized by chloride concentration (slope = -268.1, intercept = 24.3, $R^2 = 0.866$) (Figure 4, bottom). It is noted that the data points for wells 762 and 669 (circled in Figure 4) are influenced the
most by applying concentration normalization. In fact, they are the two wells with the highest and lowest sulfate concentrations (1450.2 and 101.4 mg/L, respectively, compared to an average of 575mg/L) (Table 1). Correspondingly, they also have the highest and lowest chloride concentrations (63.6 and 6.4 mg/L, respectively, compared to an average of 19.6 mg/L). Hence, the regression becomes significantly improved when concentration is normalized by chloride.

The successful use of chloride as a conservative tracer herein indicates that chloride and sulfate experience coincident concentration or dilution during evaporation or recharge, respectively. If it is true that chloride input from contaminant recharge is negligible as discussed in the Geochemistry section, such coincident concentration and dilution should have occurred after the sulfate entered groundwater (instead of in the vadose zone during infiltration because chloride is present as a background species in the aquifer). The highest sulfate and chloride concentration in well 762 may be associated with a severe drought during the 1950s and 1960s in Arizona (Nace and Pluhowski, 1965; McPhee et al., 2004). In such a case, the drought may have significantly reduced the volume of water in the aquifer by evapotranspiration. The low sulfate and chloride concentration in well 669, which is located just to the east of the western outcrop area (groundwater recharge area), can be explained by greater volumes of recharge experienced at its location.

In summary, the positive relationship between sulfur isotope composition of sulfate and sulfate concentration, in combination with unfavorable redox conditions, excludes the possibility of bacterial sulfate reduction. The linear relationship between
δ^{18}O and δ^{34}S of sulfate, plus the linear relationship between δ^{34}S and the inverse of sulfate concentration normalized by chloride concentration, indicates the presence of a two-source mixing process. The successful use of chloride as a conservative tracer indicates coincident concentration or dilution of sulfate and chloride within the aquifer.

Quantification of Sulfate Sources

The robust linear relationship between δ^{18}O and δ^{34}S (Figure 2) provides an opportunity to quantify the contribution of each sulfate source with a relatively high degree of confidence using the dual-isotope end-member mixing analysis presented in the Data Analysis section. In this study, the two identified sources of sulfate are (1) sulfuric acid applied during ore processing and (2) sulfate generated from sulfide-mineral oxidation in the tailing piles. The sulfuric acid is no longer obtainable and the tailings where the sulfide minerals reside have all been removed. Hence, direct measurement of the isotopic composition of either source is impossible. However, reasonable estimates are possible.

Wells 601 and 602 are background wells located upgradient of the source zone. It can be assumed that they were not affected by sulfuric acid use. But their location (south of the source zone) is where the bedrock is exposed, hence they should have received sulfate generated from sulfide-mineral oxidation of the bedrock. This is reflected in their sulfate concentrations (~ 100 mg/L), which are greater than the true background concentrations (~ 20 mg/L) obtained from well 402 located upgradient of the source zone but not in the outcrop area (Table 1). Isotopic composition values for groundwater from
wells 601 and 602 ($\delta^{18}O = 3 \, \text{‰}, \delta^{34}S = -11.5 \, \text{‰}$) are within the range of sulfate isotope values for sulfide-mineral oxidation (Krouse and Mayer, 1999) and lies on the extrapolated regression line to the bottom left (Figure 2). It is assumed that these values represent the isotopic composition of the source associated with sulfide-mineral oxidation. For the sulfuric acid source, its $\delta^{34}S$ is determined to be $24.3\%$ from the y-intercept of the $\delta^{34}S$ vs Cl/SO$_4$ plot (Figure 4, bottom). The background sulfate concentration is ignored in the calculation since it is generally much lower compared to concentrations associated with contaminated wells.

There are uncertainties associated with the estimation of the isotopic values of these two sources. The uncertainty associated with the value for sulfate from sulfide-mineral oxidation should be small. Moreover, this value is already very close to the upper limit of sulfate generated from sulfide-mineral oxidation (Figure 2). It provides an upper limit for the estimation. The true value can only be more negative. This provides an upper limit for the estimation of the contribution from sulfide-mineral oxidation. The uncertainty associated with the isotopic value for the sulfuric acid is related in part to the fact it was obtained by linear regression. However, the results are in fact not sensitive to the end-member estimates. For example, if we reduce the $\delta^{34}S$ of sulfuric acid by two units to $22.3\%$, the calculated source concentration of sulfate originating from sulfuric acid (except well 762) changes from approximately 120 to 100 mg/L. Such relatively small changes will not significantly influence the overall results.

The resultant concentrations and proportions of each source for each well are listed in Table 2 and visualized in Figure 5. It is shown that the percent of sulfate from
sulfuric acid is more than 70% for all but four wells. Sulfate concentrations associated with sulfuric acid varies significantly from 44 – 1152 mg/L whereas sulfate generated from sulfide-mineral oxidation is generally much lower but steady (~ 117 mg/L) for all but one well, 762, which had a sulfate concentration from sulfide-mineral oxidation equal to 542 mg/L. This high concentration is hypothesized to be associated with concentration of groundwater due to evapotranspiration that may have occurred during the major drought in the southwest U.S. during the 1940s through mid-1960s, as mentioned above. Despite this high sulfate concentration from sulfide-mineral oxidation, the majority of its total sulfate is still derived from sulfuric acid (908 mg/L). Considering the dispersion and dilution this distal part of the plume experienced during transport, the initial sulfate concentration was likely relatively high.

Contrary to our original conceptual model in which the closer hot spot was caused by release of sulfate in the source zone by sulfide-mineral oxidation, well 771 (representing the closer hot spot) is marked by the highest percentage (94%) of sulfate from sulfuric acid. This indicates that a source of sulfuric acid persisted at the site for at least approximately 40 years. Given the climatic conditions at the site, it is likely that sulfate salts (formed from neutralization of sulfuric acid) accumulated in the tailing pile and surface soil during the mill operation. Rainwater would likely dissolve some of the salts, and subsequent infiltration would serve as a long term source of sulfate. The rainfall in this area is not only sparse but also unevenly distributed (mostly in the summer monsoon and winter period), meaning that the leaching would likely be periodic rather than continuous.
Results of prior studies conducted at the site support the hypothesis of sulfate-salt formation. Before the removal of the tailings, tailing solutions were collected using lysimeters implemented up to 6 m below the tailing pile (DOE, 1999). Sulfate concentration as high as 4510 mg/L were observed. After the removal of the tailings, subpile soils were collected (at locations where tailing piles were previously located) and leached with DI water. Leachable sulfate concentrations as high as 9190 mg/kg of soil were reported (DOE, 1999). Both data sets indicate the accumulation of large quantities of sulfate salts in the tailing piles and subpile soils. Based on the isotopic analyses reported herein, these sulfate salts appear to have originated primarily from the sulfuric acid that was used during mill operation. The formation of sulfate salts from neutralization of sulfuric acid and subsequent evaporation and condensation of the salts should exhibit negligible isotope fractionation (e.g., Thode and Monster, 1965; Raab and Spiro, 1991). Thus, the isotopic composition of the sulfate salts is expected to be the same as that of the original sulfuric acid, which was estimated to be 24.3‰ based on the y-intercept of the $\delta^{34}\text{S}$ vs Cl/SO$_4^-$ plot.

To further elucidate the role of chloride in this study, the sulfate from sulfide-mineral oxidation was normalized by chloride for each well and listed in Table 2. As mentioned in the previous section, for the linear relationship between isotopic composition and inverse concentration to be valid, the concentration of the first source (sulfide-mineral oxidation) needs to be constant. Table 2 shows that it is evident that the sulfate concentration from sulfide mineral oxidation is in fact not constant (147.4 ± 116 mg/L), especially for wells 762 (542 mg/L) and 669 (58 mg/L). However, after the
sulfate concentration is normalized by chloride concentration, the values are much more constant (7.5 ± 1.1, 5.8 for well 762 and 9.0 for well 669), hence making the linear correlation much stronger (Figure 4). This indicates that chloride is successfully serves as a conservative tracer. Thus, it can be used as an indicator for concentration (evapotranspiration) or dilution (recharge and dispersion) effects. For wells 762, the solutes were concentrated by approximately 63.6/18.4 = 3.5 times. For well 669 solutes were diluted by approximately 18.4/6.4 = 3 times.

Finally, quantification of sulfate sources is performed using the method that plots isotopic composition relative to the inverse of concentration (the mass-conservation mixing method presented in the Data Analysis section). This method is further extended by the key equation $\text{slope} = \frac{C_1}{C_i} (\delta_1 - \delta_2)$. Using the slope of -268.1 (Figure 4 bottom), estimates of $\delta_1$ (-11.5 ‰) and $\delta_2$ (24.3 ‰), and the average chloride concentration of 19.6, the representative sulfate concentration for the first source is estimated to be 146.8 mg/L. This value is in good agreement with the 147.4 mg/L calculated using the dual-isotope end-member mixing approach (Figure 2). In fact, using the same equation $\text{slope} = \frac{C_1}{C_i} (\delta_1 - \delta_2)$ and the individual chloride concentration for each well, it is possible to quantify each sulfate source. The results are listed in Table 2. The two methods yielded very comparable results (Figure 6).

**Conclusions**

A combined geochemical and isotopic approach was used to characterize the sources of the sulfate plume at a former uranium mill tailing site. The contributions of the
two primary sources typically identified in prior studies, sulfuric acid and sulfide-mineral oxidation, were quantified using two methods, which yielded similar results. The results indicated that sulfide-mineral oxidation has been a minor but constant source of sulfate (~117 mg/L) compared to sulfate derived from sulfuric acid, which has been a major source with highly variable but persistent input (44-1152 mg/L). Chloride served as a conservative tracer useful for characterizing the history of evapotranspiration and dilution of the plume.

It is hypothesized that sulfate salts formed after neutralization of sulfuric acid and accumulated in the source zone. These salts appear to have served as a persistent and significant source for sulfate contamination of groundwater. Such accumulation is likely made possible by the arid climate. The methods used in the study allowed identification and quantification of the contribution of this process, which may be significant but not delineated for other sites.

**Acknowledgements**

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Table 1: Groundwater geochemistry from 14 contaminated wells and 5 background wells. “-” means no measurement.

Concentrations are in mg/L except for Al, Mn, and Fe (μg/L).

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Contaminated

| Avg±Stdv | 3.9±3.2 | 7.4±0.2 | 105±39.1 | 85.5±36.4 | 89.0±84.1 | 10.6±12.2 | 12.1±3.3 | 17.3±27.8 | 19.2±39.1 |

Background

| Avg±Stdv | 2.2±2.3 | 8.0±0.3 | 21.2±7.0 | 28.8±12.2 | 80.4±24.9 | 2.8±0.5 | - | 26.4±35.4 | 30.0±67.1 |
Table 1 continued

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Contaminated

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Background
Table 2 Sulfate isotopes of each well and estimated fraction and sulfate concentrations from each sulfate source. Subscripts T, sulfuric, and sulfide designate total, sulfuric acid, and sulfide-mineral oxidation, respectively. $C_{\text{sulfuric}}$ and $C_{\text{sulfide}}$ are calculated using the dual isotope approach and $C'_{\text{sulfuric}}$ and $C'_{\text{sulfide}}$ are calculated using the isotope composition vs inverse concentration approach.

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<th>$\delta^{18}$O (‰)</th>
<th>% $C_{\text{sulfuric}}$</th>
<th>% $C_{\text{sulfide}}$</th>
<th>$C_{\text{sulfuric}}$ (mg/L)</th>
<th>$C_{\text{sulfide}}$ (mg/L)</th>
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Figures

Figure 1 Sulfate concentration distribution in 2008. Note 2008 data was used because this data set is more complete. The sampling (geochemistry and isotopes) in this paper was done in 2009 and the concentrations are comparable to 2008. Well 728 through 731 are not shown in the figure since their position and concentration are very close to well 765.

Figure 2 $\delta^{18}$O vs $\delta^{34}$S for all contaminated wells (circles). Also shown are ranges of isotopic composition of sulfate from different sources (Krouse and Mayer, 1999). The two diamonds represent isotopic composition of the two end-members (top right assumed to represent sulfuric acid, bottom left assumed to represent sulfide-mineral oxidation). The presumed sulfuric acid end-member resides in the isotope composition range for evaporites, probably due to its production from evaporites.

Figure 3 $\delta^{34}$S of sulfate relative to sulfate concentrations.

Figure 4 $\delta^{34}$S of sulfate relative to the inverse of sulfate concentration (top) and relative to the inverse of normalized (by Cl) sulfate concentration (bottom).

Figure 5 Concentrations of sulfate derived from sulfuric acid and sulfide-mineral oxidation relative to the distance from the source. The dashed line at 117 mg/L delineates the average concentration for sulfate generated from sulfide-mineral oxidation excluding
well 762, which had abnormally high sulfate concentration from sulfide-mineral oxidation.

Figure 6 Correlation between the results calculated by the two methods. \( C_{\text{sulfuric}} \) is the sulfate concentration derived from sulfuric acid calculated by the dual isotope method. \( C'_{\text{sulfuric}} \) is the sulfate concentration derived from sulfuric acid calculated by the isotope vs inverse concentration method.
Figure 2

\[ \Delta^{18}O_{SO_4} \text{ vs. } \Delta^{34}S_{SO_4} \]

- Atmospheric Deposition
- Evaporites
- Soil Sulfate
- Sulfide Mineral Oxidation
- Slope ~ 1:4
Figure 3
Figure 5
Figure 6

The graph shows a linear relationship between $C_{\text{sulfuric}}$ (mg/L) and $C'_{\text{sulfuric}}$ (mg/L) with the equation $y = 1.0108x$ and $R^2 = 0.9938$. The data points are well-aligned with the regression line, indicating a strong correlation.
APPENDIX C: TRANSPORT AND FATE OF AMMONIUM AND ITS IMPACT ON URANIUM AND OTHER TRACE ELEMENTS AT A FORMER URANIUM MILL TAILING SITE

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

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Abstract

The remediation of ammonium-containing groundwater discharged from uranium mill tailing sites is a difficult problem facing the mining industry. The Monument Valley site is a former uranium mining site in the southwest US with both ammonium and nitrate contamination of groundwater. In this study, samples collected from 14 selected wells were analyzed for major cations and anions, trace elements, and isotopic composition of ammonium and nitrate. In addition, geochemical data from the U.S. Department of Energy (DOE) database were analyzed. Results showing oxic redox conditions and correspondence of isotopic compositions of ammonium and nitrate confirmed the natural attenuation of ammonium via nitrification. Moreover, it was observed that ammonium concentration within the plume area is closely related to concentrations of uranium and a series of other trace elements including chromium, selenium, vanadium, iron, and manganese. It is hypothesized that ammonium-nitrate transformation processes influence the disposition of the trace elements through mediation of redox potential, pH, and possibly aqueous complexation and solid-phase sorption. Despite the generally relatively low concentrations of trace elements present in groundwater, their transport and fate may be influenced by remediation of ammonium or nitrate at the site.

Keywords

nitrification, ammonium, nitrate, mobilization, uranium, heavy metals, mill tailing, groundwater
Introduction

The United States experienced a boom in uranium mines (OECD/IAEA, 2011) from 1947 through the 1970s. Due to a lack of waste-management protocols, extensive contamination has resulted at these sites. As of 1999, under the Uranium Mill Tailings Remedial Action (UMTRA) program, DOE completed the surface remediation for 24 uranium mill processing sites, with a total cost close to 1.5 billion dollars (Rael et al., 1999). However, the US Environmental Protection Agency (EPA) estimates that there are 4000 mines with documented uranium production, and another 15,000 locations with uranium occurrences in 14 western states (US EPA, 2012). The Navajo Nation alone has more than 500 abandoned uranium mines (US EPA, 2008).

Ammonium, nitrate, and sulfate are common groundwater contaminants observed at uranium mining sites. Ammonia is used in large quantities for the precipitation of uranium as “yellowcakes” (ammonium diuranate) during ore processing. Nitrate-containing reagents are generally used in limited quantities, yet extensive nitrate contamination in groundwater has often been observed at uranium mining sites (Landa, 2004). This nitrate appears in many cases to have formed via a microbial nitrification process in the subsurface (e.g., Ivanova et al., 2000). Conversely, denitrification has also been observed at uranium mining sites (e.g., Longmire and Thomson, 1992). Sulfate typically originates from use of sulfuric acid, and in some cases the oxidation of sulfide minerals associated with waste rock and mine tailings.

In addition to ammonium, nitrate, and sulfate, uranium and a host of heavy metals and metalloids (termed trace elements henceforth) are often present at uranium
mining sites. Management of groundwater contamination at such sites has focused primarily on the remediation of NH$_4$ and/or NO$_3$ (e.g., Johnson and Humenick, 1980; Ivanova et al., 2000). In contrast, the geochemical interaction between nitrogen species (ammonium and nitrate) and trace elements (including uranium) has not received much attention. In fact, ammonium and nitrate have several important geochemical properties that may have significant implications for the transport and fate of uranium and other trace elements.

First, ammonium and nitrate are redox regulators (e.g., Christensen et al., 2001). Aerobic oxidation of ammonium consumes oxygen and hence helps to maintain less-oxidative redox conditions (e.g., Christensen et al., 2000). Conversely, nitrate is a relatively strong oxidant in the redox ladder. Therefore, the presence of ammonium or nitrate has implications for the immobilization of certain redox-sensitive elements such as uranium, chromium, and arsenic. For example, the results of a recent pilot-scale injection test showed that the introduction of nitrate to a reducing zone where reduced, immobilized U(IV) resided caused remobilization of the uranium (Wu et al., 2010). Furthermore, the control of ammonium and nitrate on redox conditions also has great influence on the concentration of dissolved organic carbon (DOC). DOC is an excellent ligand for certain heavy metals and thus has potential significant implications for their stabilization and facilitated transport.

A second means by which ammonium can influence trace elements is through mediation of pH. Ammonium is a weak acid (NH$_4^+$ $\rightarrow$ NH$_3$ + H$, K_a = 5.6 \times 10^{-10}$), consequently its presence lowers the pH. In addition, the oxidation of ammonium to
nitrate produces protons (\( \text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{H}^+ \)), producing additional acidity in the groundwater. The pH not only affects the speciation (e.g. pH/Eh) of metals in solution, but also affects the adsorption of many metals on solid surfaces through its impact on solid-phase surface charge.

Third, ammonium is a surface sorption competitor, and thus can compete for cation exchange sites on solid surfaces with heavy metals. The following series of relative selectivity has been presented in order of decreasing affinity for cation exchange sites (e.g., Domenico & Schwartz 1998): \( \text{Al}^{3+} > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{NH}_4^+ > \text{K}^+ > \text{H}^+ > \text{Na}^+ \).

Fourth, ammonia and nitrate are ligands for aqueous metal complexation. Ammonia (\( \text{NH}_3 \)), the neutral molecule, which is in equilibrium with \( \text{NH}_4^+ \) in the aqueous phase, is an excellent ligand for certain heavy metals. Nitrate, as an anion, is also a good ligand for metal complexation.

The Monument Valley DOE site is a former uranium mill tailing site at which groundwater is contaminated by both ammonium and nitrate. The objective of this study is to use stable isotopes of ammonium and nitrate in conjunction with geochemical data to characterize the transformation between ammonium and nitrate. The role of ammonium and nitrate on the disposition of several trace elements is then evaluated by assessing the relationships between their respective concentrations obtained from a site survey comprising 14 wells across the plume.
Site Background

The Monument Valley site is located within the Navajo Nation in northeastern Arizona, 24 km south of Mexican Hat, UT. Uranium mining at the site occurred from 1943 to 1968, after which the mill closed (Dawson, 1992). During that time the site was one of the largest producers of uranium in the state of Arizona (with a production of 767,166 tons of uranium and vanadium ore). From 1964 to 1968, batch leaching and heap leaching were used to process an estimated 1.1 million tons of tailings and low-grade ore at the site (DOE, 2005). Sulfuric acid solution was used for both leaching processes. After leaching, ammonia and quicklime (calcium oxide) were used to produce a bulk precipitate of concentrated uranium and vanadium. Mill tailings were deposited in unlined tailing piles on site. Under the Uranium Mill Tailings Remedial Action (UMTRA) program, the US Department of Energy (DOE) has completed the surface remediation of this site by 1994 and transferred it to the Long-Term Surveillance Plan (LTSP), which conducts long-term inspections and groundwater monitoring at the disposal sites. Analysis of subpile soil samples (samples collected from beneath the “footprint” of the former tailings piles) indicates that these soils may be a continuing source of groundwater contamination. Ammonium in the subpile soil appears to be contributing to nitrate contamination in ground water (DOE, 2005).

The shallow alluvial aquifer is comprised of well-sorted fine to medium sand deposits interspersed with finer silt and clay sediments. Generally, the alluvial deposits range from 1 to 35 meters in thickness, with the greatest depths observed in the center of the valley. Depth to groundwater is approximately 11 meters. The screened intervals for
most monitoring wells span approximately 15 to 25 m below ground surface. The mean hydraulic gradient is approximately 0.01, with a northeastward direction. Hydraulic conductivities range from 0.1 to 5.6 m/day for the alluvial aquifer (DOE, 1999). The sediments have relatively low iron (~ 1500 mg/kg) and manganese oxide content (~ 30 mg/kg), and low clay and organic-carbon contents.

Groundwater geochemistry within the contaminated plume area is Ca-Mg-Na-SO$_4$-CO$_3$ type. Ca, Mg, and Na concentrations are quite comparable, with values ca. 105, 86, and 89 mg/L, respectively. Sulfate concentration is high, with a median value of 575 mg/L. Alkalinity as CaCO$_3$ is approximately 220 mg/L on average. Nitrate and ammonium concentrations are also high and comparable, with median values of 101 and 75 mg/L as N, respectively.

**Materials and Methods**

In this study, 14 wells across the entire plume area were selected for detailed geochemical and isotopic analyses. Groundwater samples were collected using dedicated bladder pumps and a QED Micropurge controller. Sampling began once field parameters (pH and dissolved oxygen) stabilized. Samples were first collected for measurement of basic field parameters (dissolved oxygen, pH, oxidation-reduction potential, and temperature), then for analyses of nitrogen species, major cations and anions (Na, Mg, K, Ca, Mn, Fe, Cl, and SO$_4$), a suit of trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, and Pb), N and O isotopes of nitrate, and N isotopes of ammonium (only a few isotope samples were collected for ammonium). Samples for
nitrogen species were collected in 500-mL HDPE bottles. Samples for cations and anions were filtered (0.45 μm) and 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 isotope analysis. These samples were filtered (0.45 μm) and collected in separate HDPE bottles of various sizes depending on the concentration. For ammonium and sulfate isotope samples, hydrochloric acid was added to pH < 2 for preservation. All samples were stored on ice in the field then frozen upon returning to the lab to curtail any additional fractionation.

Major cations and trace elements were analyzed using a Perkin Elmer ELAN DRC-II ICP-MS (inductively coupled plasma mass spectrometry), following US EPA Method 6020. Quantification limits for major cations (Na, K, Ca, Mg, Al) are mostly better than 10 μg/L. Quantification limits are 1 μg/L for Zn and Se, 0.5 for Fe, 0.2 for Mn, and approximately 0.1 μg/L for the others. Major anions (nitrate, nitrite, bromide, and sulfate) were analyzed using a Dionex ICS-1000, following EPA standard method 300.0. \( \text{NH}_4^+ \) analysis was done using a Hach colorimetric kit and analyzed using a Hitachi U-2000 Spectrophotometer. Quantification limits are 0.1 mg/L for \( \text{NO}_3^- \), \( \text{NO}_2^- \), F\(^-\), Br\(^-\) and \( \text{NH}_4^+ \) and 0.5 mg/L for Cl\(^-\) and \( \text{SO}_4^{2-} \). A low range calibration curve was used for very low \( \text{NH}_4 \) concentrations, which allowed concentration as low as 0.01 mg/L to be quantified. DOE routinely analyzes groundwater samples collected from most of the wells on site for ammonium, nitrate, sulfate, other major cations and anions, DO, ORP, pH, radionuclides, uranium, and selected other metals. Some of the DOE data (especially
uranium data) were used where current analysis is not available or not complete. The reported quantification limit for uranium is less than 0.1 μg/L.

Stable isotope analysis of ammonium and nitrate has been demonstrated to be very useful for characterizing the occurrence of nitrification or denitrification (e.g., Kendall and Aravena, 2000) and will be used in this study. Both nitrification and denitrification induce significant fractionation, meaning the product will have a much lower ratio of heavier isotopes than the reactant. Nitrification can be differentiated from denitrification by comparing N isotopes of NH₄ and NO₃ given that nitrification affects the isotopic composition of NH₄ but denitrification does not. Analysis of nitrate isotope samples was conducted at the University of Waterloo Environmental Isotope Laboratory. Ammonium isotopes were analyzed at the Boston university stable isotope laboratory. Nitrogen ($^{15}$N/$^{14}$N) and oxygen ($^{18}$O/$^{16}$O) isotope ratios are reported in delta (δ) per mil (‰) units relative to the international reference materials atmospheric nitrogen and V-SMOW, respectively. Analytical precision was 0.5 ‰ for δ$^{15}$N in NO$_3$ or NH$_4$ and 0.6 ‰ for δ$^{18}$O in NO$_3$.

**Results and Discussion**

**Ammonium and Nitrate Transformation**

Ammonia was used in the ore processing, which caused direct ammonium contamination of groundwater (ammonia is converted to ammonium following acidification). Nitrate (in the form of ammonium nitrate) was also used in the milling process. However, the quantity used is much smaller compared to ammonia (DOE, 2005).
Both contaminants are now observed in high concentrations in groundwater. Ammonium and nitrate concentrations range between 0 to 230 and 0 to 190 mg/L as N, respectively. Figure 1 shows the concentration distribution of ammonium and nitrate. Concentrations within the source zone are low (within a few mg/L), reflecting the effectiveness of the surface remediation in the source area.

The concentration distribution is different for ammonium and nitrate. The ammonium plume extends only to the center of the plume (defined by well 648, concentration = 9.2 mg/L as N), whereas nitrate comprises the full extent of the plume (e.g., well 762 concentration = 130 mg/L as N) (Figure 1). Furthermore, a general trend of decreasing ratio of NH$_4$/NO$_3$ over distance is observed along the cross-section AA’ (Figure 2), which is taken in the middle of the plume (Figure 1), except for the first data point (well 606) which is closest to the source zone and whose value is probably influenced by the surface remediation. This difference can be attributed to either retardation or nitrification of ammonium, or some combination thereof. It is difficult to distinguish between the effects of retardation and nitrification based solely on the ammonium and nitrate concentration data.

Stable isotopes of ammonium and nitrate were used to further investigate the fate of ammonium. The N isotope compositions of NH$_4$ and NO$_3$ plotted relative to the ratio of ammonium to total nitrogen (NH$_4$ + NO$_3$) are shown in Figure 3. First of all, the δ$^{15}$N of NH$_4$ is approximately 12‰ greater than the δ$^{15}$N of NO$_3$. Second, both δ$^{15}$N of NH$_4$ and δ$^{15}$N of NO$_3$ increase as the ratio of ammonium decreases. This is consistent with the isotopic effect of a nitrification process. The data is matched well by the regression
employing Rayleigh fractionation, with an initial δ^{15}N of 6‰ and an enrichment factor of -8‰ (Figure 3). The enrichment factor (-8‰) is consistent with literature-reported values ranging between -5 and -35‰ (Aravena and Mayer, 2009).

The good fit produced by the Rayleigh equation does not necessarily indicate that nitrification is the sole process influencing ammonium fate. For example, there may be uncertainties in the two underlying assumptions for the Rayleigh fractionation analysis: 1. retardation of ammonium has not disturbed the correspondence of ammonium and nitrate, 2. nitrate is solely from nitrification (i.e. nitrate applied during mill operation is negligible). However, the assumptions may be relatively robust given the conditions at the site. The sediments are relatively homogeneous sands with relatively low metal-oxide contents, and low clay and organic-carbon contents. Furthermore, the aqueous concentrations of cation-exchange competitors, Ca, Mg, and Na, are relatively high (Table 1). Therefore, retardation of ammonium is likely relatively small. In addition, the quantity of nitrate used during mill operation was relatively small, and it is likely that this nitrate entered the subsurface in the early stages of plume development (thus residing in the leading edge of the plume). Therefore, the nitrate within the major part of the plume most likely was generated from nitrification. It is difficult to fully evaluate the validity of these assumptions. However, the fact that δ^{15}N of NH₄ is much greater than δ^{15}N of NO₃ and the trend of increasing δ^{15}N with decreasing NH₄ ratio are strong lines of evidence for nitrification.

The oxygen isotope composition of nitrate was also examined (Figure 4). Values range from -1.81 to 6.96‰, with most of the values between ±3‰. No general trend was
observed when it is plotted against remaining ammonium ratio. Oxygen isotope fractionation for nitrate is controlled by the oxygen isotope composition of the oxygen sources involved in the process. Traditional theory states that the nitrification of ammonium to nitrate involves incorporation of a total of three oxygen atoms, two from H₂O and one from atmospheric oxygen. Atmospheric oxygen has a δ¹⁸O value of +23.5‰ (Kroopnick and Craig 1972) and most groundwater has a δ¹⁸O value of approximately -10‰ (e.g., Clark and Fritz, 1997). Thus, the resultant nitrate should have a δ¹⁸O value close to 0‰. However, field values slightly higher than those theoretical predictions have been reported and have been attributed to ¹⁸O enrichment in H₂O due to evaporation (Wassenaar, 1995; Kendall and McDonnell, 1998). In this study, the tailing solution was stored in open impoundments under arid climatic conditions, where severe evaporation is expected. The observation that most of the δ¹⁸O values of nitrate are within ±3‰ is consistent with nitrification of ammonium.

The redox conditions of the aquifer are generally suitable for nitrification, with DO levels typically more than 1 mg/L. However, values below 1 mg/L are observed at localized zones. The results of previous studies, employing stable-isotope analysis, reactive-transport modeling, and laboratory microcosm experiments, indicated that denitrification was occurring at this site at a slow rate (Carroll et al., 2009; Jordan et al., 2008). Given the results presented above, it is concluded that nitrification of ammonium is the primary process influencing the disposition of ammonium in the subsurface, and that nitrification and denitrification are jointly impacting nitrate fate.
Relationships between Ammonium and Trace Elements

Trace element concentrations in the groundwater contaminant plume are relatively low. Trace elements include uranium (U), chromium (Cr), selenium (Se), vanadium (V), iron (Fe), manganese (Mn), nickel (Ni), cobalt (Co), zinc (Zn), arsenic (As), and barium (Ba) (Table 1). Figure 5 shows the observed relationships between concentrations of the trace elements and those of ammonium. The relationships will be discussed by classifying them into three types of behavior.

The first type of observed behavior is an inverse relationship. Such a relationship was observed for uranium, chromium, selenium, and vanadium (Figure 5, a, b, c, and d). Uranium concentrations ranged from 5 – 85 μg/L (MCL = 30 μg/L). The highest uranium concentrations were observed for the cases wherein ammonium concentrations were approaching non-detect. It is well known that microbial reduction of the soluble, oxidized form of uranium, U(VI), to insoluble U(IV) is an important mechanism for the immobilization of uranium in the subsurface (e.g., Lovley et al., 1991). In this case, it is hypothesized that ammonium, a reducing agent, sustains the reducing condition in the aquifer, hence keeping uranium immobilized. The impact of ammonium on the redox condition is manifested by the relationship between ammonium and dissolved oxygen (DO) (Figure 6a). Dissolved oxygen is observed to be inversely related to the ammonium concentration, reflecting that the consumption of oxygen by nitrification is the primary mechanism that controls the redox. In addition, organic carbon (measured by TOC), which is an indicator for reducing conditions, also exhibits a direct relationship with ammonium concentration (Figure 6b). In the complex subsurface environment, there are
other processes such as aqueous complexation (with $\text{CO}_3^{2-}$, $\text{SO}_4^{2-}$, $\text{OH}^-$ and organic matter) and sorption that can influence the mobility of uranium (Bernhard et al., 1998). However, the influence of redox conditions appears to have the greatest impact on uranium disposition for the study site.

Chromium, selenium, and vanadium also exhibited inverse concentration correlations with ammonium (Figure 5, b, c, and d). The common property among these three is that their oxidation state is sensitive to redox conditions, and they all have higher mobility in the higher oxidation state, similar to uranium. For example, Cr(VI) compounds are highly soluble, mobile, and bioavailable compared to the sparingly soluble Cr(III) compounds. Similarly, selenates ($\text{SeO}_4^{2-}$) are more soluble compared to selenite ($\text{SeO}_3^{2-}$), and vanadium exists primarily in oxidation states 3-5 and the higher the oxidation state, the more soluble they are (Cornelis, 2005).

It is worth noting that stronger linear relationships are observed for Cr and Se compared to U and V (Figure 5, a, b, c, and d). However, the relationship for U is improved significantly by excluding the three data points that have the highest ammonium concentrations (> 100 mg/L as N). This might indicate an enhanced metal mobility, possibly by an enhanced aqueous complexation with either organic matter (TOC) or $\text{NH}_3$, at high ammonium concentration. For V, the relationship is impaired by the two data points associated with the highest and lowest V concentrations. The potential significant sensitivity of the relationships for Cr, Se, and U concentrations is suggested by the linearity observed even at lower concentrations (0.01 – 1 mg/L) of $\text{NH}_4$. 
The second type of behavior is a direct correlation between element concentration and ammonium concentration. Such behavior was observed for iron (Figure 5e) and manganese (similar to Fe, data not shown), which are commonly used for assessing groundwater redox conditions. They are also sensitive to redox conditions, but in contrast to U, Cr, Se, and V mentioned above, they speciate from solid-phase oxides to aqueous-phase cations under reducing conditions. Again their concentrations are hypothesized to be related to ammonium concentration through the effect of ammonium on redox condition. In addition, studies have shown that ammonium can serve as an electron donor for direct oxidation by manganese oxides (Hulth et al., 1999), the net result of which would increase the aqueous concentration on Mn. In both cases (ammonium maintaining a reducing condition or direct oxidation of ammonium), an overall increased element concentration with increased ammonium concentration is expected.

The third type of behavior is a slight or no apparent correlation between element and ammonium concentrations. This class includes Ni, Co, Zn, As, and Ba (Figure 5 f, g, and h). Unlike those trace elements mentioned in the first two classes, all of the elements in this class except As have relatively stable oxidation states. Therefore, their concentrations are not affected by redox conditions, and thus not influenced by ammonium concentrations in the manner discussed above.

It is known that the aqueous concentration of Ni is dependent on pH in the presence of metal-oxide surfaces, whose adsorption capacity is mediated by pH (e.g., Cornelis, 2005). Figure 6c shows the relationship between Ni concentration and pH, which is consistent with the observed increase in sorption with increasing pH typically
reported in the literature. Interestingly, it is observed that pH may correlate negatively to ammonium concentration for concentrations greater than 0.1 mg/L (Figure 6d), most likely because NH$_4$ acts as a weak acid. Thus, a positive correlation between Ni and ammonium can be established excluding the points with relatively small ammonium concentrations (Figure 5f). Cobalt exhibits behavior similar to that of nickel (data not shown). Zn, which may be expected to exhibit similar behavior as Ni and Co, however, showed no such correlation to ammonium (Figure 5g).

The distribution of arsenic between the solution and solid phases is expected to be dependent on redox condition, given that the solubility of its reduced form is higher. However, arsenic is present as an oxyanion in aqueous solution, which means that its sorption would typically increase with decreasing pH. As shown above, higher ammonium concentration is associated with lower pH (enhanced sorption for As), and stronger reducing conditions (higher solubility for As). It is hypothesized that the two competing processes rendered the arsenic concentration in effect uncorrelated to ammonium concentration (Figure 5h). Finally, barium concentration (data not shown) is controlled by sulfate concentration, according to the saturation indices of barium sulfate (close to saturation). Thus, its behavior is not influenced by ammonium concentration.

Conclusions

Nitrification was confirmed to be occurring at this site. The consumption of oxygen by ammonium oxidation will lead to reducing conditions in the aquifer. It is hypothesized that this impact on redox conditions influences the fate of several trace
elements, specifically uranium, chromium, selenium, vanadium, iron, and manganese. Ammonium also appears to have a small impact on pH, and consequently is speculated to influence the adsorption capacity of solid-phase metal oxides and thus the distribution of nickel and cobalt.

The exhaustion of ammonium (complete conversion to nitrate) will eventually result in an aerobic aquifer. In fact, a general trend of increasing nitrate and decreasing ammonium has been observed for some wells over the past decade. In addition to the nitrification process, the distribution of ammonium is also influenced by plume migration. Such transitions in ammonium and nitrate distributions will fundamentally alter subsurface conditions (redox, pH) and thereby may influence the distribution of certain trace elements. For example, for the trace elements in the first class (U, Cr, Se, and V), such a transition in redox status could result in their mobilization in the subsurface.

In-situ remediation of ammonium- or nitrate-contaminated groundwater typically involves an oxidation step (ammonium to nitrate) and/or a reducing step (nitrate to nitrogen gas) (e.g., Patterson et al., 2004; Nooten et al., 2008). When such a remediation strategy is used, the potential of influencing the mobility of certain trace elements should be considered. For example, a pilot-scale ethanol injection test was conducted at the same site to investigate the efficacy of nitrate remediation by biositimulation (Borden et al., 2012). It was observed that after the injection, uranium concentration in groundwater collected from the injection well decreased by a factor of 30 over time (Figure 7). Conversely, arsenic concentration increased more than fivefold. Both changes are due to
the change in redox condition from oxic to reducing conditions induced by addition of the
electron donor and concomitant increase in microbial activity (enhanced denitrification).

Acknowledgements

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References


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Table 1 General geochemistry and trace element concentration of the 14 sampled wells. Element concentrations are all µg/L. Concentrations of other constituents are mg/L. NH$_4$ and NO$_3$ are as N. Alkalinity is as CaCO$_3$. U and alkalinity are from the DOE database. ND means non-detected.

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Figures

Figure 1 Site map showing source zone (the fenced area), well locations, and NH$_4$ and NO$_3$ concentrations at each well.

Figure 2 Change of NH$_4$/NO$_3$ ratio relative to the distance from the source zone along the cross-section AA’.

Figure 3 $\delta^{15}$N of ammonium and nitrate vs the ratio of remaining ammonium which represents the nitrification progress. Data are represented by square symbols and calculation results are represented by curves. The calculation used an initial $\delta^{15}$N of ammonium equals to 6‰ and an enrichment factor of -8 ‰.

Figure 4 $\delta^{18}$O of nitrate relative to ratio of remaining ammonium. Dashed lines delineate the range of ±3 ‰.

Figure 5 Trace element concentrations relative to ammonium concentration. Regression in plot a and f excluded the circled data.

Figure 6 DO, TOC, and pH vs ammonium concentration and Ni concentration vs pH. Figure 7 Change of uranium and arsenic concentrations over time after biostimulation.
Figure 1
Figure 2

The graph shows the ratio of NH₄/NO₃ against distance (meters). The data points are plotted along with a fitted curve that demonstrates a decreasing trend as the distance increases.
Figure 5

(a) $y = 13.66x^{0.32}$  
$R^2 = 0.69$

(b) $y = 0.41x^{0.43}$  
$R^2 = 0.91$

(c) $y = 2.86x^{0.24}$  
$R^2 = 0.87$

(d) $y = 11.74x^{0.09}$  
$R^2 = 0.19$

(e) $y = 1.35x^{0.02}$  
$R^2 = 0.49$

(f) $y = 0.03x^{0.04}$  
$R^2 = 0.68$

(g) $Zn$ (μg/L)

(h) $As$ (μg/L)
Figure 6

(a) DO (mg/L) vs. NH₄ (mg/L) with the equation $y = 3.55x^{0.117}$ and $R^2 = 0.34$

(b) TDOC (mg/L) vs. NH₄ (mg/L) with the equation $y = 1.04x^{0.33}$ and $R^2 = 0.56$

(c) Ni (µg/L) vs. pH with the equation $y = 7E+06e^{-1.9x}$ and $R^2 = 0.46$

(d) pH vs. NH₄ (mg/L) with the equation $y = -0.15\ln(x) + 8.10$ and $R^2 = 0.78$
Figure 7
APPENDIX D: THE IMPACT OF BIOSTIMULATION ON THE FATE OF SULFATE AND ASSOCIATED SULFUR DYNAMICS IN GROUNDWATER

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ABSTRACT

The impact of biostimulation on the fate of sulfate and associated sulfur dynamics in groundwater was investigated during a field project conducted at a former uranium mining site. Geochemical data and stable-isotope data for sulfate collected before and after the test were analyzed to evaluate the sustainability of sulfate reducing conditions induced by the tests, 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. After the injection, the occurrence of bacterial sulfate reduction was confirmed by an exponential decrease of sulfate concentration in concert with changes in ORP, redox species, sulfide, alkalinity, and fractionation of δ$^{34}$S-sulfate. Sulfate reducing conditions have been maintained for a period of approximately 3 years. A Rayleigh fractionation model was used successfully to simulate the measured data, with estimated intrinsic enrichment factors ranging from -5 to -9‰. Atypical fractionation behavior of the δ$^{34}$S in sulfate was hypothesized to be caused by release of sulfate from sulfate minerals associated with the sediments. The Rayleigh fractionation simulations incorporated an incrementally increasing degree of sulfate-mineral dissolution as the sulfate reduction process proceeded, and the estimated contribution of mineral dissolution was approximately 10% of the initial sulfate concentration. Elevated hydrogen sulfide concentrations were not observed until approximately four months after the start of the test. This behavior, in concert with the observed changes in aqueous iron and manganese species, suggests that hydrogen sulfide produced from sulfate reduction was precipitated, presumably in the form of iron sulfides,
until the exhaustion of readily reducible iron oxides. Hydrogen sulfide produced thereafter appears to have been in part re-oxidized. The results of this study illustrate the complex biogeochemical behavior that is associated with the in-situ biostimulation process for sulfate reduction, and suggest that the success of such a remediation approach depends on the mineralogy of the aquifer, aqueous geochemistry, and other site conditions. A large fraction of sulfate was reduced and sequestered in this study. The long-term stability of the precipitates formed during a biostimulation effort needs further investigation.

**Keywords**: sulfate reduction, stable isotopes, isotopic fractionation, iron sulfide precipitation, sulfate mineral dissolution, sulfide re-oxidation
INTRODUCTION

Sulfate contamination of groundwater has become a recent environmental concern. However, due to its highly soluble and stable nature, the remediation of sulfate in the subsurface is a difficult proposition. Microbially induced reduction, as has been employed to address contaminants such as nitrate, is one option for sulfate. However, in contrast to denitrification, the product of sulfate reduction, hydrogen sulfide (H\textsubscript{2}S), is undesirable due to its toxicity. To improve the viability of this approach, the reduction product (H\textsubscript{2}S) needs to be stabilized in the solid phase, possibly in the form of metal sulfides. Hence, bacterial sulfate reduction is commonly used for remediation of acid mine drainage because the precipitation of heavy metal sulfides effectively mitigates both heavy metal and sulfate concentrations (e.g.,).

Heavy metals are typically present at only trace levels in most sulfate plumes, and thus are not a viable precipitation agent. However, it has been observed that bacterial sulfate reduction in aquifers often involves the precipitation of iron sulfides using indigenous iron oxides. This route of precipitation may hold promise for successful in-situ remediation of sulfate-contaminated groundwater via bacterial sulfate reduction in aquifers that have moderate iron contents.

Sulfur and oxygen isotopes in sulfate have long been used to characterize the fate of sulfate in subsurface systems. However, the use and interpretation of isotope data is often confounded by other processes. For example, it has recently been reported that secondary processes such as metal-sulfide precipitation, hydrogen-sulfide re-oxidation, metal-sulfide re-oxidation, and dissolution of sulfate-bearing minerals can affect the
isotopic signature of the aqueous sulfate pool. Therefore, isotope data should be used in combination with aqueous and sediment geochemistry data for a sound understanding of the system.

Two pilot-scale ethanol-injection tests were recently conducted to investigate the efficacy of biostimulation for remediation of nitrate-contaminated groundwater at a former uranium mining site. The objective of the research reported herein is to investigate the impact of the biostimulation efforts on the fate of sulfate and associated sulfur dynamics. Geochemical data and stable-isotope data for sulfate collected before and after the test were analyzed to evaluate the sustainability of sulfate reducing conditions induced by the tests, 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. Uranium mining at the site occurred from 1943 to 1968. In the leaching processes, sulfuric acid solution was used, causing direct groundwater sulfate contamination. Tailings were stored at two tailing piles without protection, causing long-term sulfate generation from pyrite oxidation. The unconfined alluvial aquifer was contaminated with high levels of nitrate (another chemical used during ore processing) and sulfate. The contaminant plume emitting from the source zone to the northeast for at least 2 km. Concentrations are as high as 170 mg/L (as N) and
1500 mg/L for nitrate and sulfate, respectively. Heavy metal contamination of groundwater is minimal within the study area. Natural attenuation for nitrate and sulfate was reported to be slow at this site due to the generally oxidative nature of the aquifer.\textsuperscript{12} Previous laboratory microcosm studies using sediments from the site indicated that ethanol could significantly enhance the rate of denitrification.\textsuperscript{12, 13}

The sediments at the site comprise a red-brown, very fine sand, with minimal clay and organic-carbon content.\textsuperscript{14} Generally, the alluvial deposits range from 1 to 35 meters in thickness, with a water table depth of approximately 11 m. The mean hydraulic gradient is approximately 0.01, with a general northeastward direction. Hydraulic conductivities range from 0.1 to 5.6 m/day for the entire alluvial aquifer.\textsuperscript{14} The mean groundwater pore water velocity is estimated to be 44 m/y.

\textbf{Test Procedures}

The study area is within the middle of the plume (550 m to the source zone) where sulfate concentrations are intermediate (600 mg/L) and have been stable over several years. The study area comprises a 3 m × 12 m plot in which nine monitoring wells reside (Figure 1). The screened intervals for the monitoring wells span approximately 15 to 25 m below ground surface. Sulfate concentrations were uniform (~600 mg/L) among all wells prior to the tests. Two ethanol-injection tests were conducted. Test 1 was conducted using the single-well push-pull method at well 765 (Figure 1). This method was selected to constrain the area of influence, which allowed comparative monitoring of sulfur dynamics between the treatment zone and the downgradient unaffected area.
Approximately 2850 L of groundwater was extracted and mixed with 150 L ethanol (5% by volume) in a sealed inflatable bladder. In addition, 300 g of potassium bromide was added to serve as a conservative tracer. The solution was injected into well 765 with a flow rate of 3.8 L/min. Upon completion of the solution injection, an additional 600 L of groundwater (free of ethanol and bromide) was injected to flush the ethanol solution out of the borehole and into the surrounding aquifer. After the injection, a 48-hour incubation period was implemented, after which groundwater was extracted from the well at a rate of 3.8 L/min for a period of 48 hours to withdraw the ethanol solution. Test 2 was conducted using the standard single-well injection method, with well 729 serving as the injection well (Figure 1). The single-well injection test was conducted one year after the first test when all monitored parameters were restored to background conditions prior to the test for all wells except well 765. Groundwater was extracted and mixed with ethanol to form a 1% ethanol solution, which was injected in well 729 for 80 hours with a flow rate of 3 L/min. A lower ethanol concentration was used based on the results observed for the push-pull test. Detailed test procedures and information about breakthrough curves for ethanol and bromide are reported in Borden et al.\textsuperscript{10}

**Sampling and Analytical Methods**

Groundwater samples were collected from all wells at the test site prior to, during, and after both tests. Samples were collected using dedicated bladder pumps and a QED Micropurge controller. Sampling began once field parameters (pH and dissolved oxygen) stabilized. Samples were collected for basic water chemistry parameters (dissolved
oxygen, pH, oxidation-reduction potential, and temperature), nitrogen species, major cations and anions (Na, Mg, K, Ca, Mn, Fe, Cl, and SO₄), ethanol, hydrogen sulfide (H₂S), and sulfate stable isotope (δ³⁴S) 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 ³⁴S isotope of sulfate was 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. Calibration was linear in the range -10 to +30 per mil. Analytical precision was estimated to be ± 0.15‰ or better (for 1 standard deviation), based on repeated internal standards. Isotopic composition of sulfur in sulfate
was reported in delta notation. The enrichment factor was obtained from the slope of the linear regression for the semi-log plot of $\delta^{34}\text{S} \text{ vs } C/C_0$ for each sampling location. The initial sulfate concentration ($C_0$) and isotopic composition ($\delta^{34}\text{S}_0$) were obtained in a background sampling round prior to the tests.

**Sediment Characterization**

Subsurface sediment samples were collected when the boreholes for monitoring wells 731 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 0.1%. 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 for Fe, 0.2 for Mn, and approximately 0.1 µg/L for the others. DOE conducted sulfate leaching tests for 26 sediment samples collected from both the source zone and a background uncontaminated area (500 to 1000 m upgradient of the source zone). 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 shows 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 there is a moderate amount of iron associated with the sediment for potential iron-sulfide precipitation.

The results of sediment-leaching tests conducted by the DOE for background (uncontaminated) sediment samples showed water-extractable sulfate concentrations ranging from 138 to 458 mg/kg, with acid-extractable sulfate ranging from 107 to 158 mg/kg. For contaminated sediments, water-extractible sulfate ranged greatly from 25 mg/kg to thousands of mg/L and acid-extractible sulfate ranged from 70 to 180 mg/kg. These results indicate that sulfate-bearing minerals are present in the sediment. 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

Test 1 involved injection and withdrawal of ethanol solution in well 765 (Figure 1). Nitrate was reduced from approximately 200 mg/L as nitrogen to less than 1 mg/L within 25 days (data not shown). Following depletion of nitrate, sulfate concentrations
began to decrease exponentially (Figure 2), with a first-order rate coefficient (k) of 0.005 d\(^{-1}\) \((R^2 = 0.997)\). In addition to nitrate and sulfate concentrations, changes in other parameters related to redox conditions were observed. Comparing samples collected before and five months after the ethanol injection, ORP decreased from 75 mV to -150 mV and dissolved oxygen decreased from 2.4 to 0.6 mg/L, consistent with the development of reducing conditions. Concentrations of dissolved iron and manganese increased from 1 and 3.8 μg/L to 300 and 60 μg/L, respectively. This is attributed to the use of iron and manganese oxides as electron acceptors for ethanol transformation and their associated reduction to more soluble iron and manganese cations. Finally, alkalinity increased from approximately 250 to 800 mg/L as CaCO\(_3\). The increase of alkalinity is attributed to production of HCO\(_3^-\) from oxidation of ethanol. The changes observed for all of these parameters are consistent with the occurrence of bacterial sulfate reduction. Sulfate concentrations for the injection well decreased to less than 50 mg/L after approximately 1 year, and remained at that level for approximately two years thereafter (Figure 2, last two observations not shown), indicating long-term sustainability of the sulfate reducing condition induced by a single input of ethanol.

For test 2, the ethanol solution was injected in well 729 and migrated to the downgradient wells (Figure 1). The rate of decrease in dissolved sulfate concentrations observed for the injection well \(k = 0.007 \text{ d}^{-1}\) was similar to that observed for test 1 \(\text{d}^{-1}\) (Figure 2). Decreases in sulfate concentration were observed after approximately one month for two downgradient wells 730 and 743 (Figure 2). Interestingly, the rates of decrease were greater for these two wells \((k = 0.018 \text{ d}^{-1} \text{ and } 0.020 \text{ d}^{-1}, R^2 = 0.952 \text{ and } \)
0.978, respectively) compared to the injection well. This is most likely because the injection well continuously received fresh groundwater with abundant sulfate whereas the downgradient wells did not (or at least to a lesser extent). The decrease in sulfate concentration for well 741 occurred later and at a slower rate (Figure 2). Geochemical changes (ORP, DO, Fe, Mn, and alkalinity) observed for those wells that were influenced by the injection are similar to those observed for test 1.

**Disposition of Hydrogen Sulfide**

Production of hydrogen sulfide exceeding background was observed coincident with decreasing sulfate, albeit at concentrations (~20-750 μg/L) significantly below levels stoichiometrically equivalent to sulfate reduction (Figure 3). A dramatic increase in dissolved hydrogen sulfide (>10,000 μg/L) was observed after approximately 70 days (Figure 3). These high concentrations are approximately representative of stoichiometric (1:1) conversion of sulfate to hydrogen sulfide. This is in distinct contrast to conditions present during the initial stage of the test, wherein hydrogen sulfide concentrations were much lower. This disparity indicates that a significant fraction of the hydrogen sulfide generated during the early stage of the test was sequestered in some manner. Based on the moderate iron content of the sediments, this sequestration is hypothesized to have occurred through the formation of iron-sulfide precipitates (e.g., $FeS_2$), wherein the source of the dissolved iron was generated through use of iron oxides as electron acceptors for transformation of ethanol. The significant latter-stage increase observed for hydrogen sulfide concentration is hypothesized to result from exhaustion of readily reducible iron.
Based on a mass-balance analysis, assuming that all sulfide equivalent represented by the difference between sulfate reduced and sulfide measured occurs as iron-sulfide precipitates, it is estimated that the equivalent of 300 mg/L of sulfate was precipitated as iron sulfide in the early stage of the test in the injection zone. Using a similar mass-balance analysis, it is estimated that the equivalent of approximately 85 mg/L of sulfate was reduced to and released as un-sequestered hydrogen sulfide in the latter stage of the test. In addition, estimates of precipitated concentration of 360 mg/L (sulfate equivalent) and released concentration of 60 mg/L (H₂S, sulfate equivalent) were obtained for well 730, and 320 mg/L precipitated and 60 mg/L released were obtained for well 743. In aggregate, the analyses indicate that the equivalent of approximately 300 mg/L sulfate was reduced and precipitated before hydrogen sulfide concentrations increased significantly. This translates to approximately 50% percent of the original sulfate.

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., ⁹). This amount of iron represents approximately 1 - 2% of the total acid digestible (5N HNO₃) iron measured for the sediment from the study site. The results of prior research indicate that only a portion of total iron associated with iron oxides is reducible, depending upon degree of crystallinity.¹⁵ Thus, it is reasonable to hypothesize that the availability of reducible iron became limiting at some point in the system given the moderate iron-oxide content associated with the sediment.
**Stable Isotope Analysis**

The $\delta^{34}\text{S}$ of sulfate increased with a decrease in sulfate concentration during test 1 (data not shown) and the initial stage of test 2 (Figure 3). Due to the slightly different reactivity of the lighter and heavier molecules of sulfate (e.g. $^{32}\text{SO}_4$ vs $^{34}\text{SO}_4$), biochemical reactions preferentially consume the lighter molecules ($^{32}\text{SO}_4$), leading to enrichment of the heavier isotope ($^{34}\text{SO}_4$) in the residual sulfate. Hence, bacterial sulfate reduction will result in enriched $^{34}\text{SO}_4$ (denoted as an increase in $\delta^{34}\text{S}$) for the residual sulfate in concert with decreases in sulfate concentration. This process is termed isotopic fractionation and is often represented with a Rayleigh distillation model.

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 test 2 (Figure 3). The points at which the $\delta^{34}\text{S}$ of sulfate begin to decrease correspond to the points at which the sulfate concentrations have decreased to values that are much lower than initial. One possible process that could cause the observed decrease is the release from the sediment phase of sulfate that is less enriched than the extant aqueous sulfate. Based on the results of the sediment characterization, there is a significant pool of sulfate associated with the sediment in the form of sulfate-bearing minerals that can be released upon a change in geochemical conditions. One potential mechanism for sulfate release is the dissolution of relatively soluble primary sulfate minerals triggered by the decrease of sulfate concentration induced by sulfate reduction. Another potential mechanism may include release of sulfate impurities from the dissolution of non-sulfate minerals such as calcite.
and dolomite induced by reaction of these minerals with carbonic acid produced during biostimulation. The mixing of the sediment-associated sulfates with the residual original aqueous-phase sulfate will lead to a decrease in the $\delta^{34}S$ of the mixed aqueous-sulfate pool if the sediment-phase sulfate is less enriched than the residual original aqueous-phase sulfate.

The isotopic composition of the sulfate in the plume area is distinct from the background due to the impact of sulfate generated from sulfuric acid used during mill operation. The aggregate isotopic signature of the sulfate minerals can be estimated from the signature of aqueous sulfate in the uncontaminated areas of the site, assuming equilibrium between aqueous and sediment-phase sulfate. Several samples collected from several background wells showed a mean $\delta^{34}S$ value of -9.4‰. This significantly $^{34}$S-depleted value indicates that the sulfate associated with the sulfate-bearing minerals is much less enriched in $^{34}$S than the residual original aqueous-phase sulfate within the test area, which has experienced significant enrichment due to the impact of bacterial sulfate reduction. This is consistent with the hypothesis that the decrease in $\delta^{34}S$ observed in the latter stage of test 2 is a result at least in part of sulfate-mineral dissolution. The effect of sulfate dissolution on the isotopic signature of the total sulfate pool would be most prominent when the sulfate concentration is low due to a greater proportion of sulfate from mineral dissolution.

The decrease in $\delta^{34}S$ of sulfate observed in the latter stage of test 2 could potentially also be explained by the oxidation of hydrogen sulfide back to sulfate (termed sulfide re-oxidation), which may have occurred during the latter stage of the test. Because
the sulfide produced from sulfate reduction is enriched in the lighter isotope compared to the sulfate pool, its re-oxidization back to sulfate (re-oxidation induces negligible isotopic fractionation) and subsequent mixing with the extant sulfate pool would reduce the aggregate isotopic signature. As discussed above, the majority of hydrogen sulfide produced from sulfate reduction was apparently sequestered via precipitation of iron sulfides during the initial stage of the test. However, this sequestration greatly diminished in the latter stage, and hence the concentration of hydrogen sulfide in solution increased greatly. This un-sequestered sulfide would be expected to be much more susceptible to be oxidized back to sulfate.

Significant increases in the concentration of manganese in groundwater (from ~1 to more than 3000 μg/L) were observed for the latter stage of test 2 (Figure 3). The times at which the concentrations begin to increase significantly correspond to the occurrence of significant increases in hydrogen sulfide. It is hypothesized that the increased levels of hydrogen sulfide caused enhanced manganese oxide reduction (i.e., anoxic oxidation of hydrogen sulfide). Anoxic oxidation of sulfide by manganese oxides has been previously reported.\textsuperscript{16-21} This re-oxidation process, given that it proceeds under reducing conditions, can occur simultaneously with continued sulfate reduction. Another contributor could be diffusive fluxes of dissolved oxygen and nitrate associated with fresh groundwater surrounding the reaction zone.\textsuperscript{22-24} It is difficult to evaluate the degree of influence of sulfide re-oxidation on the $\delta^{34}$S signature of the aqueous sulfate pool, but it is likely to be small given that manganese concentrations were in the 3 mg/L range (compared to sulfate concentrations in the hundreds of mg/L) and that diffusive fluxes are usually small under
a stable hydraulic field. Based on this analysis, it is concluded that release of sulfate from the sediment phase was of greater significance to the atypical isotope behavior observed for the latter stage of test 2.

The occurrence of sulfide re-oxidation is more evident for test 1, wherein oxidizing conditions prevailed throughout the entirety of the test. For test 1, a noticeable decrease in the $\delta^{34}$S-sulfate values was observed for samples collected 5 months after the injection (Figure 4) for the downgradient wells (728, 729, 730, 731, Figure 1). It is hypothesized that sulfide transported from the treatment zone (vicinity of the injection well) was re-oxidized to sulfate under the oxic conditions present in the downgradient area, which would result in a decrease in the aggregate $\delta^{34}$S of the total sulfate pool as discussed above as well as an increase in sulfate concentrations as was observed.

**Rayleigh Fractionation Modeling**

A Rayleigh fractionation model, incorporating an incrementally increasing degree of sulfate-mineral dissolution as the sulfate concentration decreases, was used to simulate the observed behavior (Figure 5). The initial aqueous sulfate pool has a $\delta^{34}$S value of 16‰, based on measurements conducted for samples collected from the study area prior to ethanol injection. The sulfate associated with sediment-associated sulfate-bearing minerals is assumed to have a $\delta^{34}$S value of -9.4‰ as reported above. Sulfate dissolution is simulated by adding a certain amount of dissolved sulfate to the aqueous sulfate pool after each reduction step (detailed calculations are reported in Supporting Information). For example, assuming the initial aqueous sulfate concentration is 1, in the first step of
sulfate reduction, the amount of aqueous sulfate is reduced by 0.02, e.g., from 1 to 0.98. Then, a certain amount (e.g. 0.001) of sulfate dissolves from the sediment phase into the aqueous phase. The total amount of aqueous sulfate becomes 0.98 + 0.001 = 0.981 and the resultant $\delta^{34}$S of the mixed sulfate pool ($\delta_{\text{mix}}$) is calculated using a simple mass balance equation: $\delta_{\text{mix}} = (C_1\delta_1 + C_2\delta_2) \div C_T$. Then, the resultant mixed sulfate undergoes additional sulfate reduction, after which additional sulfate is produced via mineral dissolution. It is assumed for these simulations that the dissolution of sulfate increases at each step due to the decreasing total sulfate concentration. An arithmetic increase is used for simplicity.

Three simulated curves are shown in Figure 5, corresponding to three scenarios in which enrichment factor $\varepsilon = -5\%$ and total amount of dissolved sulfate $D_t = 0.14$, $\varepsilon = -5\%$ and $D_t = 0.10$, and $\varepsilon = -9\%$ and $D_t = 0.10$, respectively. Also shown in the figure for comparison are two curves with no dissolution ($\varepsilon = -5\%$ and $\varepsilon = -9\%$, respectively). It is manifest that the dissolution of sulfate-bearing minerals for which the sulfate is less enriched suppresses the increase of $\delta^{34}$S in the aqueous sulfate pool due to bacterial sulfate reduction, rendering the apparent enrichment factor much smaller in magnitude than the actual (inherent) enrichment factor. The effect of sulfate-mineral dissolution is more evident at the latter stage of the event (shown by the inflection points of the curves in the figure) because dissolution is enhanced at lower aqueous concentrations and hence the fraction of sulfate from the sediment phase becomes relatively greater compared to the total aqueous sulfate when the remaining aqueous sulfate is low. The simulations provide reasonable representation of the measured data, especially capturing the pattern
of decreasing $\delta^{34}\text{S}$ at low $f$ (fraction residual sulfate). The curve with the smaller enrichment factor and larger amount of dissolution ($\varepsilon = -5\%$ and $D_t = 0.14$) fits the data for wells 730 and 743 (two downgradient wells) quite well. Conversely, the other two curves with smaller amount of dissolution encompass most of the data points for the two injection wells 765 and 729. This difference is reasonable given that sulfate concentrations declined to significantly lower levels in the downgradient zone compared to the injection zone.

Enrichment factors of -5 and -9‰ were obtained from the simulations presented above. These values are somewhat smaller than values reported in the literature, which typically range from -10‰ to -20‰. The magnitude or extent of fractionation is expected to be dependent upon the magnitude and rate of microbial activity. Thus, the enrichment factor may be expected to be smaller when electron donor is available in abundance. 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‰). The condition of non-limiting substrate supply was satisfied with the abundant carbon substrate used in our study (5% and 1% ethanol solution for tests 1 and 2, respectively), 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. For example, smaller enrichment factors for bacterial sulfate reduction were observed in experiments using ethanol as the carbon substrate compared to other carbon sources. 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.\textsuperscript{31}

**IMPLICATIONS**

The impact of biostimulation on sulfur dynamics was evaluated for two pilot-scale biostimulation tests. The occurrence of bacterial sulfate reduction was confirmed by a number of parameters. Sulfate reducing conditions have been maintained for a period of approximately three years in the vicinity of the injection well. Ethanol, the original electron donor, has long since been exhausted during this period. In addition, groundwater containing nitrate and sulfate at high initial concentrations has continued to flow into the treatment zone from upgradient. The mechanism responsible for the sustained reducing conditions is unknown. However, it is speculated that one potential cause is the presence of biomass that was produced during ethanol injection that has served as a long-term carbon substrate.\textsuperscript{10} In this study, processes such as sulfate-mineral dissolution, sulfide re-oxidation, and iron-sulfide precipitation can be inferred from analysis of the aqueous geochemistry data. A Rayleigh fractionation model incorporating an incrementally increasing degree of sulfate-mineral dissolution as the sulfate reduction process proceeded successfully simulated the observed $\delta^{34}\text{S}$-sulfate data.

Despite the reduced efficiency caused by dissolution of solid-phase sulfate and possible re-oxidation of hydrogen sulfide, a significant portion of the hydrogen sulfide produced from sulfate reduction was apparently 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 mineralogy of the aquifer, aqueous geochemistry, and other site conditions. Moreover, long-term stability of the precipitates needs additional investigation.

Acknowledgements

This research was supported by the University of Arizona TRIF Water Sustainability Program through the Center for Environmentally Sustainable Mining, and the NIEHS Superfund Research Program (P42 ES04940). We thank Andy McMillan, Hakan Akyol, Andrew Borden, and Justin Berkompas for their assistance. We thank Dr. Christopher Eastoe of the Environmental Isotope Laboratory at the University of Arizona for sulfur isotope analysis.

Supporting Information

Additional information regarding the Rayleigh fractionation modeling. This material is available free of charge via the Internet at http://pubs.acs.org.
1. EPA website. Sulfate in drinking water. US Environmental Protection Agency. 
   http://www.epa.gov/safewater/contaminants/unregulated/sulfate.html

2. Miao, Z.; Brusseau, M. L.; Carroll, K. C.; Carreon-Diazconti, C.; Johnson, B., 
   Sulfate reduction in groundwater: characterization and applications for remediation. 

3. Benner, S. G.; Blowes, D. W.; Ptacek, C. J., A full-scale porous reactive wall for 
   (4), 99-107.


6. Rickard, D., Kinetics of pyrite formation by the H2S oxidation of iron (II) 
   monosulfide in aqueous solutions between 25 and 125 degrees C: The rate equation. 

7. Gibson, B. D.; Amos, R. T.; Blowes, D. W., S-34/S-32 Fractionation during 
   Sulfate Reduction in Groundwater Treatment Systems: Reactive Transport Modeling. 

   transformations in pilot-scale constructed wetland treating high sulfate-containing


Figures

Figure 1. Schematic map showing the locations of wells for the tests (test 1, injection well 765; test 2, injection well 729).

Figure 2. Sulfate concentrations for all the wells during both tests. X-axis shows the days after the injection for Test 1. Test 2 began at approximately 395 days as noted in the figure.

Figure 3. Sulfate concentrations, hydrogen sulfide concentrations, δ$^{34}$S of sulfate, iron and manganese concentrations for injection well 729 and downgradient wells 730 and 743 after test 2.

Figure 4. Comparison of sulfate concentrations and δ$^{34}$S values for injection well 765 and downgradient monitoring wells 728 through 731 before and after (5 months) test 1. The x-axis trends along a transect from up to downgradient.

Figure 5. Rayleigh distillation simulations of δ$^{34}$S evolution of sulfate undergoing sulfate reduction and sulfate release from the sediment. Also shown are the data for both tests from this study.
Figure 1
Figure 2

[Image of a graph showing sulfate concentration (mg/L) over days after the injection in well 765, with different well markers and a vertical dashed line indicating the start of injection in well 729.]
Figure 3
Figure 4

[Graph showing SO₄ (mg/L) and δ³⁴S (%) for samples 765, 728, 729, 730, and 731, with bars indicating before and after measurements.]
Figure 5
Effect of Dissolution on $\delta^{34}$S of SO$_4$ During BSR
Supporting Information for

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

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Contents:

4 Pages

1 Table
This Supporting Information shows an example spreadsheet from excel for the calculation (simulation) of the Rayleigh fractionation process which incorporated an incrementally increasing degree of sulfate-mineral dissolution as the sulfate reduction process proceeded.

This following table shows the case when enrichment factor $\varepsilon=-5\%_o$, total dissolved sulfate $D_\varepsilon=14\%$ (increment at each step = 0.00012 relative to an initial sulfate amount of 1, number of increments = 49). $\delta^{34}S_0$ is the initial aqueous sulfate isotope composition. $\delta^{34}S_{\text{Diss}}$ is the isotope composition of the sulfate mineral in the sediment phase. $\delta^{34}S-\text{SO}_4$ is the isotope composition of the extant aqueous sulfate and $\delta^{34}S-\text{SO}_4'$ is the isotope composition of the mixed sulfate after a certain amount of sulfate has dissolved. $f$ is the fraction residual sulfate and $f'$ is the modified fraction residual sulfate after dissolution occurs. Dissolved $\text{SO}_4$ is the amount of sulfate that dissolves from the mineral phase at each step. The amount increases incrementally as the fraction residual sulfate decreases. For simplicity, the increment is a constant.

Initially (step 1), $\delta^{34}S-\text{SO}_4$ equals 16.0‰ and $f = 1$. At step 2, $f$ is force to be reduced to 0.98, when $\delta^{34}S-\text{SO}_4$ is calculated to be 16.10‰ using standard Rayleigh fractionation equation $\delta^{34}S = \delta^{34}S_0 + \varepsilon \times \ln (f)$. At this step, dissolved sulfate is assumed to be 0, so $\delta^{34}S-\text{SO}_4' = 16.10$ and $f' = 0.98$. At step 3, $f$ is forced to decrease to 0.96, when $\delta^{34}S-\text{SO}_4$ is calculated to be 16.20‰. At this step, an amount of 0.00012 (relative to an initial amount of sulfate = 1) sulfate is dissolved to the aqueous phase, mixed with the extant sulfate pool. The isotope composition of the mixed sulfate pool is calculated as $\delta^{34}S-\text{SO}_4' = (0.00012 \times -9.4\%_o + 0.96 \times -16.20\%_o)/(0.00012+0.96) = 16.2009$ and the
resulted apparent fraction residual sulfate becomes \( f' = 0.96 + 0.00012 = 0.96012 \). At step 4, the mixed sulfate pool is forced to be reduced from \( f' = 0.96012 \) to \( f = 0.94 \), when \( \delta^{34}S-SO_4 \) is calculated using the same equation \( \delta^{34}S = \delta^{34}S_0 + \varepsilon \times \ln (f) \), but now the \( \delta^{34}S_0 \) should use the \( \delta^{34}S-SO_4' \) from the last step and the fraction residual sulfate in the equation should be calculated as \( f/f' \), in this case, 0.94/0.96012. Then another amount of sulfate (0.00024) is dissolved when it is mixed with the current sulfate pool, after which another step of reduction begins. From now on, all the calculations start to repeat. The total dissolved sulfate is calculated at the bottom of the table as the sum of the dissolved \( SO_4 \) at each step.

Table 1s. Example spreadsheet from excel showing calculation (simulation) of the Rayleigh fractionation process which incorporated an incrementally increasing degree of sulfate-mineral dissolution as the sulfate reduction process proceeded. (Case when enrichment factor \( \varepsilon = -5\% \), total dissolved sulfate \( D_t = 14\% \), corresponding to the first curve in Figure 5 in the paper.)

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