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# Sulfur Isotopes as Indicators of Amended Bacterial Sulfate Reduction Processes Influencing Field Scale Uranium Bioremediation

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Aqueous uranium (U(VI)) concentrations in a contaminated aquifer in Rifle Colorado have been successfully lowered through electron donor amended bioreduction. Samples collected during the acetate amendment experiment were analyzed for aqueous concentrations of Fe(II), sulfate, sulfide, acetate, U(VI), and $\delta^{34}$S of sulfate and sulfide to explore the utility of sulfur isotopes as indicators of in situ acetate amended sulfate and uranium bioreduction processes. Enrichment of up to 7‰ in $\delta^{34}$S of sulfate in down-gradient monitoring wells indicates a transition to elevated bacterial sulfate reduction. A depletion in Fe(II), sulfate, and sulfide concentrations at the height of sulfate reduction, along with an increase in the $\delta^{34}$S of sulfide to levels approaching the $\delta^{34}$S values of sulfate, indicates sulfate limited conditions concurrent with a rebound in U(VI) concentrations. Upon cessation of acetate amendment, sulfate and sulfide concentrations increased, while $\delta^{34}$S values of sulfide returned to less than −20‰ and sulfate $\delta^{34}$S decreased to near-background values, indicating lower levels of sulfate reduction accompanied by a corresponding drop in U(VI). Results indicate a transition between electron donor and sulfate-limited conditions at the height of sulfate reduction and suggest stability of biogenic FeS precipitates following the end of acetate amendment.

## Introduction

Long-term uranium sequestration through amended microbial reduction has received much attention in recent years as a potentially fast and efficient means of groundwater remediation (*1*). In its oxidized form, U(VI), uranium exists as a highly soluble uranyl ion, $UO_2^{2+}$. In its reduced form, U(IV), uranium is insoluble and precipitates as stable minerals such as uraninite, $UO_{2(s)}$. Since enzymatic reduction of U(VI) to U(IV) was first demonstrated by Lovley et al. (*2*), over 32 strains of bacterium, mostly iron and sulfate reducers, have been identified as capable of biological uranium reduction (*1*). Subsurface microbial activity is often electron-donor limited, and thus addition of organic compounds such as lactate, acetate, ethanol, and glucose offer a straightforward means of uranium sequestration (*3*−*8*). In situ stimulated bioremediation is now a key component in the research portfolio of DOE's Environmental Remediation Sciences Program (ERSP) (http://www.lbl.gov/ERSP).

Enhanced in situ uranium bioreduction has been successfully demonstrated at several DOE remediation sites, including the Oak Ridge National Laboratory, TN and the Old Rifle Site in western Colorado (*3*−*8*). At the Old Rifle Site, acetate has been utilized as the electron donor, and the efficiency of the associated bioremediation processes has been shown to depend directly on the redox conditions generated in the aquifer. During previous acetate amendments, the highest rates of uranium removal were observed at redox levels optimal for microbial iron reduction (*3*). U(VI) bioreduction continued at lower redox conditions favoring microbial sulfate reduction but at decreased rates. Following cessation of acetate amendment and a return to higher redox conditions, uranium precipitates may be susceptible to oxidation and remobilization (*9*−*14*). However, accumulation of biogenic Fe(II) may be capable of sustaining abiotic reduction of U(VI) as oxidizing conditions return to the aquifer (*15*). Furthermore, preferential oxidation of other bioreduction products such as FeS species may aid in long-term sequestration of uranium as biogenic uraninite (*12, 15, 16*). Clearly, understanding the chemical and mineralogical changes occurring within these systems is critical to achieving maximum, sustained bioreduction of uranium.

Stable isotope studies have illustrated the benefit of using isotopic measurements to track the biological removal of contaminant species while separating out the effects of transport in through-flowing systems (*17*−*20*). Recent studies have also demonstrated enrichment of $^{238}$U relative to $^{235}$U in residual U(VI) during microbial uranium reduction (*21*). However, the use of sulfur isotopes as indicators of bioremediation progress has been limited to laboratory experiments and nonamended field-scale studies (*22*). The importance of sulfide species as products of amended bioreduction experiments suggests that stable isotopes of sulfur may prove to be an important tool in investigating in situ uranium bioremediation.

Bacterial sulfate reduction (BSR) in the subsurface can be identified through characteristic fractionations in the stable isotope compositions of sulfate and sulfide. BSR preferentially utilizes sulfate containing $^{32}$S, the lighter isotope of sulfur. This preference results in enrichment of $^{34}$S, the heavier isotope of sulfur, in the residual sulfate (*23*−*25*). The degree of enrichment of the residual sulfate pool may be influenced by a variety of parameters such as temperature and available organic substrates (*25*−*27*). U(VI) reduction by sulfate reducers has also been shown to depend on the type and variety of organic substrate, but, in general, sulfur isotope fractionation during BSR is principally dependent on the magnitude of the preference for reducing sulfate with the lighter sulfur isotope (the fractionation factor) and the extent of sulfate reduction that has occurred (*25, 28, 29*). Under most conditions, the difference between the $\delta^{34}$S of sulfate minus the $\delta^{34}$S of the sulfide produced is ≥10‰; however, under sulfate-limited conditions the fractionation factor reduces to almost zero (*25*). Precipitation of sulfide minerals

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produces an isotopic fractionation of <1‰ (*27, 30*) and will, therefore, not cause a significant change in the $\delta^{34}S$ values of the remaining aqueous sulfide even when a large fraction of the sulfide has precipitated out of solution. For these reasons, the $\delta^{34}S$ of sulfide is an excellent indicator of whether or not a system has become sulfate limited.

Sulfur isotope ratios of sulfate are also sensitive to reoxidation of sulfides, as may be expected following cessation of an amendment experiment. Oxidation of aqueous sulfide or sulfide minerals to sulfate often results in a sulfur isotope fractionation of less than 5‰ (*25*), yielding a product sulfate isotopic signature that approaches the sulfide source value. Given the large $^{32}S$ depletion associated with sulfate reduction, significant reoxidation of any sulfides should produce sulfate with anomalously low $\delta^{34}S$ values, providing an indicator of sulfur precipitate stability (*31*).

The present study is part of a 69-day acetate amendment experiment conducted at the Old Rifle Uranium Mill Tailings Remedial Action (UMTRA) site in Rifle, CO, under the ERSP. Stable isotopes of sulfur are used, in conjunction with aqueous concentrations of sulfate, sulfide, Fe(II), and U(VI), to 1) develop a better understanding of the timeline and processes influencing amended BSR and 2) elucidate the processes influencing aqueous concentrations of residual and product species of sulfur over time.

## Materials and Methods

**Field Site.** The Old Rifle UMTRA site is a small aquifer contained in an alluvial terrace deposited along a former channel in the floodplain of the Colorado River. The alluvium is approximately 6 m deep and bounded from beneath and along the north, east, and west sides by an impermeable shale layer of the Wasatch Formation (*32*). The former river channel excavated the low lying terrace along the walls of this resistant unit, such that the aquifer now sits in a crescent shaped deposit along the northern bank of the river. The mean flow gradient is 0.7−0.8 m/day toward the southwest edge of the aquifer, where groundwater discharges to the river (*3*).

The Old Rifle site was an ore-processing facility, and uranium mill tailings were left to leach into the sediments and aquifer system until surface remedial action by the DOE was completed in 1996. The tailings and the top layer of soil were removed, and the area was regraded and covered with a semi-impermeable clay layer. Aqueous U(VI) concentrations of up to 1.8 $\mu$M have since been measured near the former footprint of the tailings pile. This value exceeds the maximum UMTRA standard of 0.18 $\mu$M aqueous uranium (*32, 33*).

Multiple injection galleries have been installed in the Old Rifle aquifer to test the ability of naturally occurring bacterial communities to reduce aqueous uranium using an amended electron donor source (Figure 1). Wells were drilled to the impermeable layer at 6 m below ground surface and screened throughout the saturated profile. The large gallery (M-01 to M-15) was the first to be constructed and was used for three separate acetate-amendment experiments. These experiments demonstrated aqueous uranium removal in association with iron reduction attributed to increased activity of *Geobacter* species. As acetate amendment continued, the system shifted from iron reduction to BSR, at which point U(VI) removal rates decreased. Prolonged U(VI) removal following cessation of acetate amendments was also noted in areas where sulfate reduction had occurred, though the mechanisms of postamendment U(VI) removal have not been characterized (*3, 34, 35*). Following the success of these experiments, two additional "minigalleries" were constructed south of the larger gallery to further explore the relationships between iron and sulfate reduction and uranium removal. The samples for this study were collected during a 2006 experiment conducted in the saturated portion of the southern-most minigallery (outlined in Figure 1).

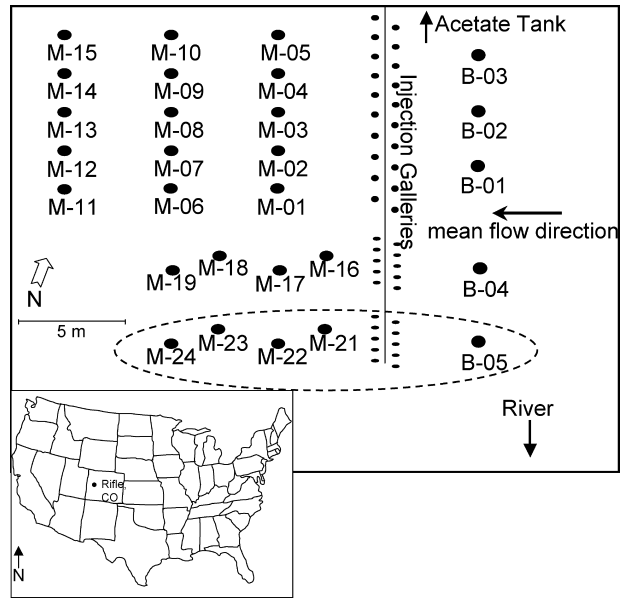


**FIGURE 1. Field site location and injection gallery/monitoring well diagram. Monitoring wells M-01 through M-15 and background wells B-03 through B-03 comprise the original or 'main' gallery (*5*). The smaller 'minigalleries" were subsequently installed for additional amendment experiments; the outlined minigallery was utilized for this research.**

**Experimental Design and Sampling.** The top of the stimulation target zone is defined by the water table (3−4 m below ground surface) and the base by the impermeable layer at the top of the Wasatch Formation (~6 m below ground surface). Tracer test, flowmeter, and pumping tests conducted within the larger main gallery directly to the north of the site suggest that the horizontal correlation length of hydraulic conductivity there is <1 m, the variance of the natural logarithm of hydraulic conductivity is high (2.1 $m^2/day^2$), and the hydrological heterogeneity influences the distribution of the injected amendment and associated transformations in this open, through-flowing site (*36*). Porosity estimates calculated from electrical well logs taken in the monitoring well gallery using Archie's relationship (*37*) indicate significant vertical and lateral geological heterogeneity and suggest preferential flow paths in this gallery.

Detailed descriptions of the well gallery design and amendment calibration can be found elsewhere (*3, 34*). Briefly, during the course of the experiment a solution of up-gradient groundwater with 10 mM sodium acetate and 1 mM potassium bromide was continuously injected at a rate of 0.1 to 0.3 L/min for 69 consecutive days to shift the system through iron and sulfate reducing conditions. The solution was mixed, sparged, held in a storage tank under a nitrogen gas headspace, and delivered to the injection wells through calibrated peristaltic pumps. Variability in the injection rate resulted from calibration and stability limitations of the pumps used to deliver the solution to the injection gallery. The injection pump array was tested prior to the start of the amendment to ensure that injection rates over this range did not influence the groundwater table elevation or mean flow path of the aquifer. Throughout the course of the natural gradient experiment, groundwater samples for chemical and isotopic analyses were collected from the up-gradient background well (B-05) and down-gradient monitoring wells (M-21 through M-24). Chemical samples were collected weekly during the injection and bimonthly following cessation of the amendment addition. For each sample set, groundwater was pumped from the designated depth(s) in

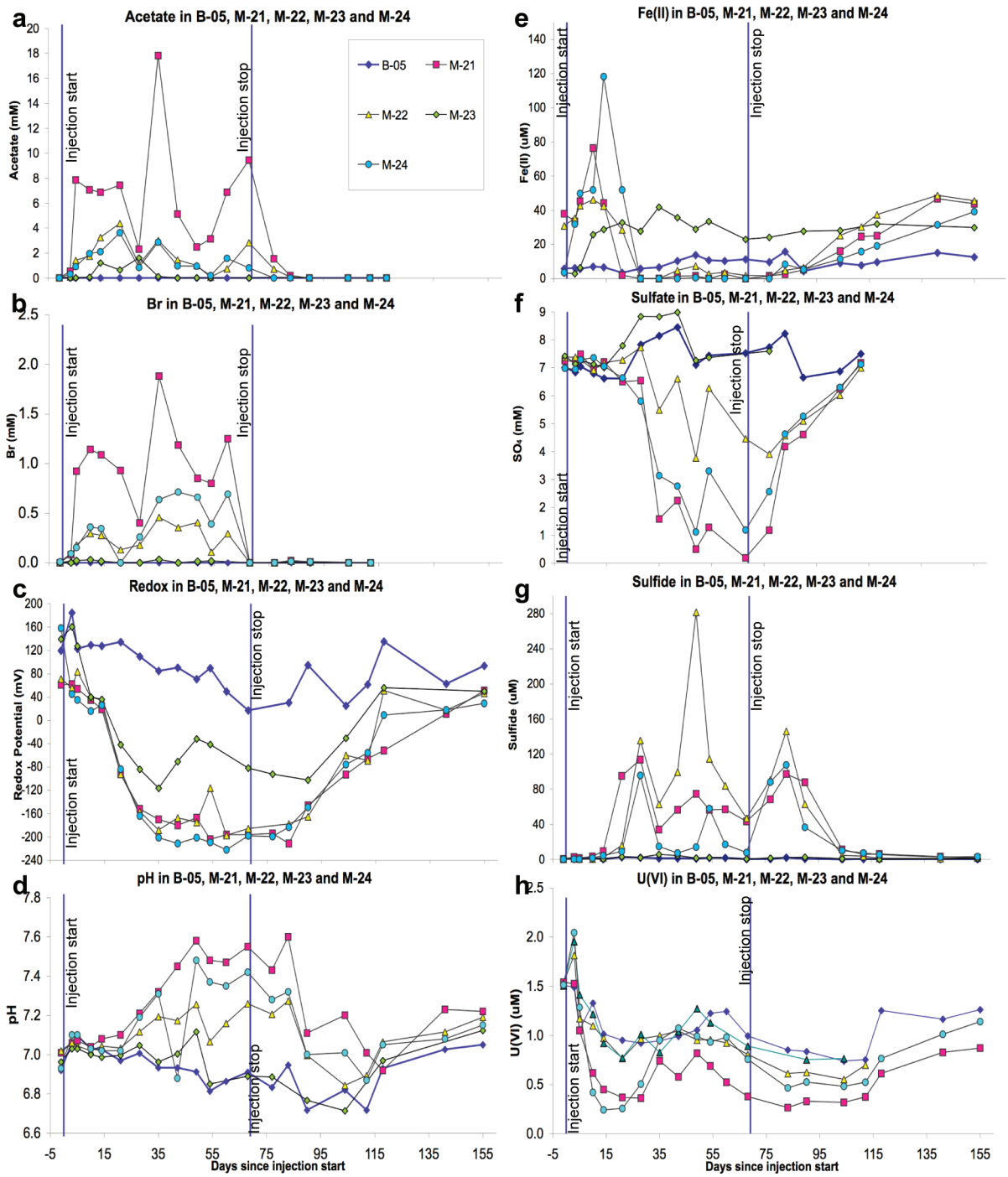


**FIGURE 2. Aqueous measurements in B-05 (background well), M-21, M-22, M-23, and M-24 of a) acetate, b) bromide, c) redox potential, d) pH, e) Fe(II), f) sulfate, g) sulfide, and h) U(VI). Values shown for B-05, M-22, and M-23 are averages of three distinct vertical depth samples collected in each well during each sampling.**

the monitoring wells using a portable peristaltic pump (ColePalmer Instrument Co.). Temperature, pH, dissolved oxygen, and redox potential were recorded until values stabilized (typically after purging 12 L at 0.5 L/min) using a calibrated multiprobe data sonde (Hydrolab Co.). Samples were then collected and filtered through a 0.2 $\mu$M PTFE syringe filter into 25 mL HDPE bottles for sulfate analyses and 15 mL plastic sterile falcon tubes for U(VI) analyses. Aqueous sulfide and Fe(II) measurements were made using a Hach spectrophotometer (Hach Co.) (*38, 39*). Samples for isotopic analysis of sulfate were filtered and reacted with acidified $BaCl_2$ to precipitate $BaSO_4$, and those for sulfide were reacted with ZnAc to precipitate ZnS (*40*). All samples were shipped from the field via overnight courier for analysis.

**Laboratory Analysis.** Uranium, sulfate, and bromide concentrations were measured at the University of Massachusetts Environmental Biotechnology Center. Uranium was analyzed by kinetic phosphorescence (*41*), while sulfate and bromide were measured on a Dionex DX-600 ion chromatograph with an AS11-HC analytical column (*38*). Sulfur isotope samples were prepared at the University of California Berkeley (*40*) and sent to the University of Nevada at Reno Stable Isotope Laboratory for analysis. Sulfur isotope analyses were performed in helium continuous flow mode, using a Eurovector model 3028 elemental analyzer interfaced to a Micromass IsoPrime isotope ratio mass spectrometer following established methods (*42, 43*), with a precision of ±0.15‰ (1$\sigma$). $V_2O_5$ was added to $BaSO_4$ samples as a

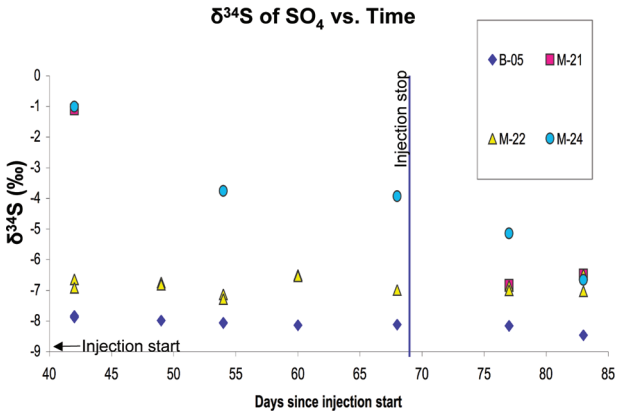


**FIGURE 3. Stable sulfur isotopes of sulfate in wells B-05, M-21, M-22, and M-24 throughout the period of sulfate reduction.**

combustion aid. Sulfur isotope ratios are reported using the conventional $\gamma$ notation

$$\delta^{34}\mathrm{S} = \left(\left(\frac{R_{sample}}{R_{std}}\right) - 1\right) \times 1000 \qquad (1)$$

where $R = {}^{34}\mathrm{S}/{}^{32}\mathrm{S}$ and the isotope ratios are reported as per mil (‰) variations relative to the internationally accepted standard Canyon Diablo Troilite (CDT).

## Results and Discussion

**Aqueous Chemistry.** Acetate amendment to the southernmost minigallery began July 13, 2006 and continued for 69 days. The concentrations of acetate and bromide measured in the monitoring wells are plotted in Figure 2 (parts a and b, respectively). The variability observed in the wells was related to refilling and mixing of the storage tank. Over the course of the experiment the redox potential in the background well (B-05) ranged from −19 to 175 mV (Figure 2c), while pH values ranged between 6.7 and 7.0 with no clear temporal trend observed (Figure 2d). Background Fe(II) averaged 9.5 mM and varied between 2.0 and 19.2 mM (Figure 2e), sulfate averaged 7.4 mM and ranged from 6.6 to 8.6 mM (Figure 2f), aqueous sulfide averaged 0.8 mM and varied from 0.0 to 3.2 mM (Figure 2g), and uranium averaged of 1.0 mM and ranged from 0.7 to 1.5 mM (Figure 2h). Temperature exhibited a typical seasonal increase from roughly 14 to 17 °C between June 5 and September 20 followed by subsequent cooling to a low of 13 °C on December 15 (Supporting Information). Although the chemical data for the groundwater samples from the background well were variable, there were no temporal trends observed. Prior to the start of the injection, values of sulfate, sulfide, U(VI), pH, and temperature in all monitoring wells were consistent with the background well values. Redox potential in M-21 and M-22 were slightly lower than in B-05, M-23, and M-24, while Fe(II) was slightly elevated, suggesting a low level of ferric iron reduction, likely as a result of previous amendment studies. Consistency of chemical data in the monitoring wells with that observed in B-05 prior to injection as well as close monitoring of water table elevations to ensure B-05, and the injection gallery remained directly up-gradient of the monitoring wells, and the success of previous studies using this well gallery configuration (*3, 34, 35*) all support the use of B-05 as an indicator of average background aqueous chemistry.

In M-21, the monitoring well closest to the injection gallery (Figure 1), the redox potential was 60 mV prior to acetate addition. After acetate injection began, the redox potential decreased slowly during the first 2 weeks and then rapidly to less than −150 mV over the next 2 weeks. It remained below −150 mV until 21 days after acetate injection stopped and then gradually increased to greater than 50 mV (Figure 2c). Fe(II) concentrations in M-21 began to rise 5 days after acetate injection started and peaked at 76 mM after 10 days before dropping to less than 3 mM by 21 days (coincident with the rapid drop in redox potential). Fe(II) remained low until acetate injection stopped and then slowly increased, reaching concentrations of 47 mM (Figure 2e). Aqueous sulfate concentrations in M-21 dropped below background 21 days after the start of injection and remained at <2.5 mM until 83 days, when sulfate began to increase, returning to background levels after 104 days (Figure 2f). Aqueous sulfide concentrations began to rise at day 10 (as Fe(II) concentrations peaked), reaching 110 mM on day 28. Coincident with the drop in sulfate concentrations, the sulfide concentration dropped to between 30 and 75 mM until day 83 when it reached a second peak value of 97 mM before decreasing to near background concentrations (Figure 2g). During the course of the amendment, pH values increased uniformly to a maximum of 7.6 (0.6 above background) before returning to background values following the course of the amendment (Figure 2d).

As noted in previous studies (*5, 36*), uranium removal in M-21 was clearly a function of the groundwater chemistry of the system (Figure 2h). U(VI) dropped below background 5 days after the arrival of acetate and concurrent with the first peak in Fe(II) and the initial rise in sulfide. Uranium decreased below 0.4 mM between days 21−28, concurrent with the first peak in sulfide concentrations and the disappearance of Fe(II). During days 28−60, the uranium concentration rebounded to between 0.5 and 1.0 mM as sulfate dropped below 2.5 mM, sulfide decreased to about half of its peak levels, Fe(II) remained near zero, and pH increased roughly 0.5 units. Upon cessation of acetate injection, the uranium once again dropped below 0.4 mM in conjunction with increases in sulfate, sulfide, and Fe(II) and a corresponding reduction to background pH values.

Several important differences are noted between the trends observed in M-21 and the chemical data from the other monitoring wells. M-24, the monitoring well furthest down gradient from the injection gallery (Figure 1), had lower acetate concentrations than M-21, but the chemical responses for redox-sensitive constituents were similar. The initial Fe(II) peak in M-24 was higher than the peak in M-21 but occurred a week later (likely due to the greater distance from the injection gallery). Sulfate concentrations in M-24 did not drop as low as in M-21 during the sulfate reduction phase, and aqueous sulfide concentrations were significantly lower. U(VI) concentrations in M-24 reached lower values than in M-21 during days 10−21 but rebounded to background levels by day 35. Starting at day 68, the U(VI) concentrations in M-24 again decreased below background, but the drop was not as large as in M-21 (Figure 2h).

In M-22, the initial peak in Fe(II) concentrations was not as high as it was in M-21 or M-24. In addition, the drop in sulfate concentrations during days 35−90 was much less (Figure 2f). Paradoxically, the sulfide concentrations in M-22 were significantly higher than in the other wells during this period, presumably as a result of lower Fe(II) production rates and correspondingly minimal removal from solution as iron sulfide precipitates (Figure 2g). At the same time, uranium removal from the groundwater in M-22 was significantly less than in M-21 and M-24, especially during the day 10−28 period (Figure 2h). These trends suggest that uranium reduction is highly sensitive to the relationship between iron and sulfate reduction.

The chemical trends in M-23 were different than in the other wells. Very little acetate and no measurable bromide reached M-23, and the redox potential did not drop as low as it did in the other monitoring wells (Figure 2c). One result of this was that Fe(II) increased more slowly than in the other wells but remained elevated (greater than 20 mM)

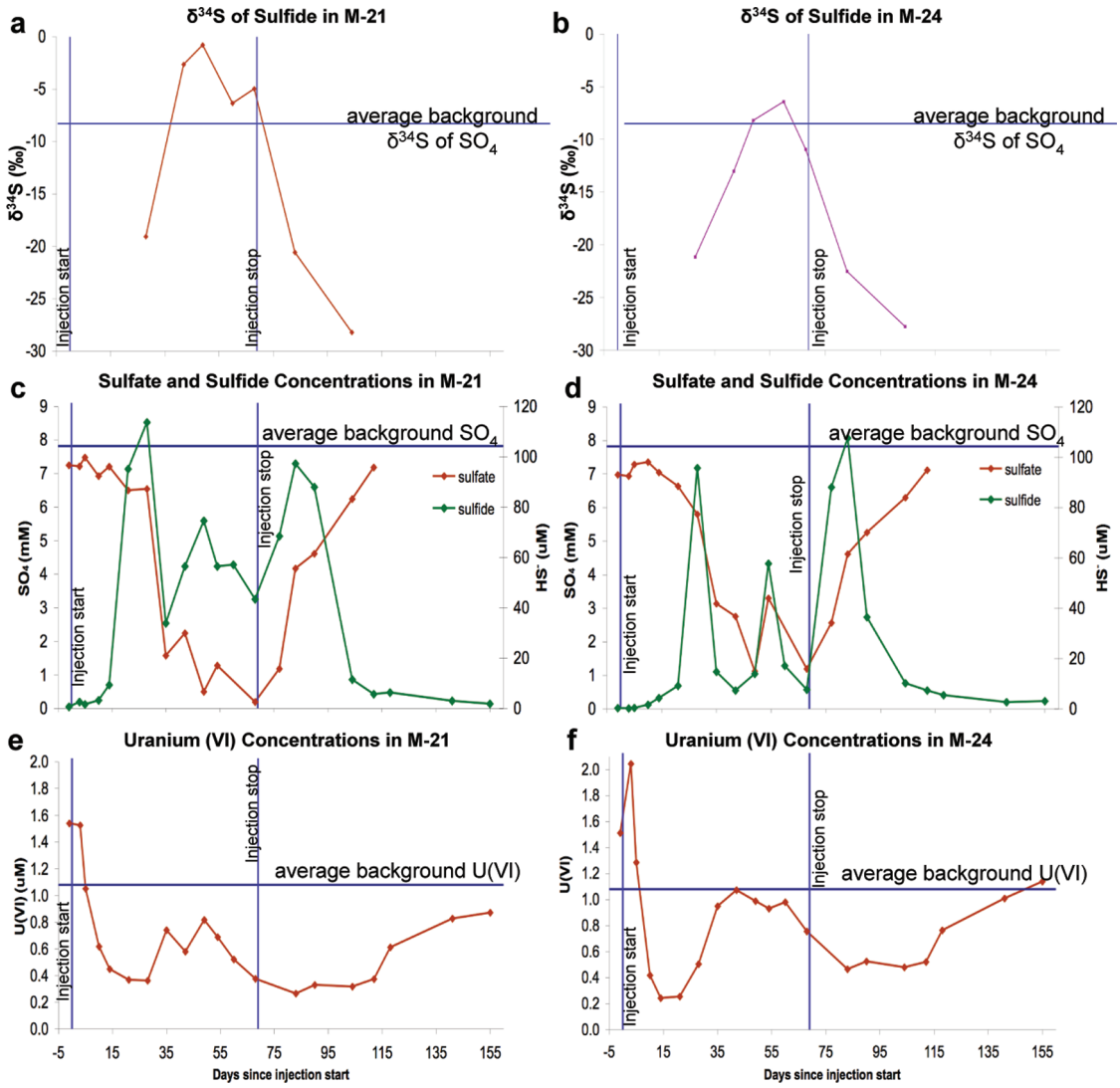


**FIGURE 4. Stable sulfur isotopes of sulfide vs time for a) well M-21 and b) well M-24. Sulfate and sulfide concentrations for c) well M-21 and d) well M-24 and uranium (VI) concentrations for e) well M-21 and f) well M-24 are also given for comparison. Background δ34S of SO4, sulfate, sulfide, and uranium (VI) concentrations are given as averages of all measurements in well B-05 throughout the course of the experiment. The background value for sulfide concentration (not labeled) is 1.25 μM.**

throughout the experiment (Figure 2e) with no indication of sulfate reduction. U(VI) concentrations remained at or near background levels despite sustained iron reduction, indicating that iron reduction alone was not sufficient to achieve significant levels of uranium removal from the groundwater.

**Sulfur Isotope Data.** The $\delta^{34}$S values of sulfate were measured in samples from B-05, M-21, M-22, and M-24 (Supporting Information). No samples from M-23 were analyzed because there was no evidence of significant sulfate reduction in this well. Samples for sulfate isotope analyses were collected from day 42 (after sulfate reduction began) through day 83 (after sulfate concentrations had rebounded to background levels and sulfide concentrations were near zero). The data are plotted in Figure 3. The sulfate $\delta^{34}$S values in well B-05 were relatively constant, averaging −8.1‰ and ranging from −7.8 to −8.5‰. Samples collected at three discrete depths in the B-05 background well showed minimal change with depth in the isotopic signature. A minor trend occurred toward more negative $\delta^{34}$S values with time.

The $\delta^{34}$S values of sulfate in M-22 averaged −6.8‰, varying from −6.5 to −7.3‰, and were consistently about 1.2‰ higher than the background isotopic compositions measured in B-05. This enrichment in the $\delta^{34}$S value of sulfate is consistent with low levels of sulfate reduction. Coupled with a minimal decrease in aqueous sulfate and low bromide concentrations, this result indicates minor levels of sulfate reduction were maintained in the area around well M-22, likely as a result of less efficient acetate delivery. This is despite the higher concentrations of sulfide maintained in M-22 throughout the acetate injection experiment, suggesting that biogenic sulfide produced in this area was not efficiently removed as mineral precipitates.

In contrast, at the peak of sulfate reduction in wells M-21 and M-24 (sulfate concentrations 0.2 and 1.2 mM, respectively) the $\delta^{34}$S values of sulfate were much more enriched than in M-22. On day 42 (the earliest date on which sulfate sulfur isotopes were analyzed), the $\delta^{34}$S values of sulfate in both M-21 and M-24 were −1.1‰. This degree of isotopic enrichment (~7‰ above background) indicates a higher degree of sulfate reduction occurred in the vicinity of these wells. During the next few sampling events, the sulfate concentrations in M-21 were too low to obtain samples sufficient for sulfur isotope analyses. In M-24, however, despite roughly constant sulfate concentrations, the $\delta^{34}$S values of sulfate showed a consistent decrease toward background values. Following cessation of acetate amendment, the sulfate concentrations and $\delta^{34}$S values in M-21 and M-24 reached levels similar to those observed in M-22 as the concentrations of sulfide increased.

The sulfur isotope ratios of sulfide were measured for samples from M-21 and M-24 beginning at the first peak in aqueous sulfide and continuing until after the second peak in sulfide following cessation of acetate amendment (Supporting Information). These data are plotted on Figure 4 with the average background sulfate $\delta^{34}$S value. Also plotted for comparison are the sulfate, sulfide, and U(VI) concentration data. In both wells, the $\delta^{34}$S values of the sulfide samples collected during the first sulfide peak (day 28) were more than 10‰ lower than the background sulfate $\delta^{34}$S values. At this time, sulfate in both wells was near background concentrations, indicating that the level of sulfate reduction was minimal despite the peaks in sulfide concentrations. The $\delta^{34}$S values of the next four sulfide samples from both wells (collected between days 42−68) were much higher than the initial samples. In M-21, the $\delta^{34}$S values were higher than the background sulfate isotope values and close to the M-21 $\delta^{34}$S values of sulfate. These samples were collected during the time period when the sulfate concentrations in M-21 dropped to below 2 mM and the sulfide decreased to approximately half of the peak concentrations. Similar trends for the sulfide isotope data from M-24 were observed, although the degree of enrichment was not as high. At the same time, the sulfate and sulfide concentrations in M-24 also dropped, but the decrease in sulfate was not as pronounced as in M-21. The small fractionation between the $\delta^{34}$S values of sulfate and sulfide (to <2‰) in these wells during this time period is a clear indication of sulfate limitation (*17, 21*). This is also the time period during which the U(VI) concentrations rebounded to near background levels (Figure 2h).

Following the cessation of acetate addition, the sulfate and sulfide concentrations in M-21 and M-24 began to increase, and the $\delta^{34}$S values of the sulfide decreased to well below background sulfate values. The last samples analyzed for sulfide $\delta^{34}$S were collected after the sulfide concentrations reached their second peaks and dropped back to concentrations of less than 10 mM. The $\delta^{34}$S values in M-21 and M-24 were −28.2‰ and −27.8‰, respectively, roughly 20‰ lower than the background sulfate isotope values and, in M-24, 21‰ lower than the measured $\delta^{34}$S value of sulfate in the same sample. Such values are anticipated during bacterial sulfate reduction when sulfate is readily available and further suggest that the initial rebound in sulfide concentrations is due to increasing levels of sulfate reduction as concentrations rebounded while acetate was still present in the system rather than reoxidation of sulfides precipitated earlier in the experiment (which would lead to a decrease in the $\delta^{34}$S values of the sulfate comparable to that observed for sulfide) (*25, 30, 31*). During this time period, the redox potential and pH in these wells began to rebound, Fe(II) concentrations increased, and U(VI) concentrations dropped.

**Implications of Chemical and Isotopic Data for Mechanism of Sulfate and Uranium Removal.** In M-23, low acetate levels correlated with a higher redox potential, sustained iron oxide reduction, and no appreciable decrease in aqueous uranium, suggesting that iron oxide reduction alone was not capable of yielding significant levels of U(VI) removal. In well M-22, low Fe(II) concentrations suggest minimal iron oxide reduction in the vicinity of this well. Minor decreases in sulfate concentrations with only slightly enriched values of $\delta^{34}$S indicate a low, sustained level of BSR. Relatively high sulfide concentrations are thus attributed to negligible FeS precipitation resulting from a lack of biogenic Fe(II) production. Near background U(VI) concentrations in this well therefore suggest acetate amended BSR alone did not lead to appreciable uranium removal rates at the Rifle site.

In wells M-21 and M-24, the highest levels of U(VI) removal occurred in association with concomitant Fe(II) and sulfide production. Microbial community analysis during this period of the amendment showed that the groundwater microbial population was still dominated by Fe-reducing bacteria (i.e., *Geobacter* spp.) though sulfide concentrations and sulfur isotopes indicate increasing BSR activity. This suggests that maximum uranium removal may have been achieved in association with FeS precipitation (*44*), a connection supported by the sulfur isotope data, though the influence of uranium desorption as a consequence of elevated pH could contribute to the subsequent rebound in U(VI) concentrations during sulfate reduction (*45, 46*). Low sulfide $\delta^{34}$S values and decreased U(VI) concentrations corresponding to the initial peak in sulfide concentrations indicate low levels BSR despite high sulfide concentrations. Isotopic values also indicate the subsequent period of low Fe(II), sulfate, and sulfide concentrations cannot be attributed to removal by FeS precipitation alone. Rather, $\delta^{34}$S of sulfide values during this time increased above background $\delta^{34}$S of sulfate and approached a null fractionation factor in M-21. This relationship is recognized as an indication of the transition to sulfate limitation (*29*), suggesting that the majority of the sulfate in the system was reduced in the up-gradient area between the injection gallery and M-21. Coupled with hydrologic data suggesting preferential flow paths across the well gallery, measurable concentrations of sulfate in M-21 (average 1.2 mM) and M-24 (average 2.3 mM) during this time are thus attributed to a combination of fast flow paths in which sulfate can pass unaffected through the reduced zone and mixing of groundwater external to the reduced zone through the sampling process. Similar effects of hydrologic heterogeneity on amendment distribution have been documented in a bioremediation experiment at the Hanford, WA site (*47*).

Following the end of the injection, the second decrease in uranium in association with increasing levels of sulfide, sulfate, and Fe(II), a decrease in pH, and a second period of high fractionation between the background $\delta^{34}$S of sulfate value and the $\delta^{34}$S of sulfide suggests a transient return to BSR conditions as the overall sulfate concentrations and $\delta^{34}$S values return to background. While the increase in sulfide and Fe(II) could be attributed to dissolution associated with the decrease in pH, observable values of sulfate $\delta^{34}$S below background level coupled with low $\delta^{34}$S for sulfide suggest there was negligible reoxidation of any high $\delta^{34}$S FeS precipitates formed during acetate amendment (*25*). Evidence for FeS stability provided by the sulfur isotope data offers implications for the stability of uranium sequestered in association with these precipitates and motivates further analysis of the effects of coprecipitation.

## Acknowledgments

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## Supporting Information Available

Figure of pH vs time and tables of sulfate and sulfide $\delta^{34}$S values. This material is available free of charge via the Internet at http://pubs.acs.org.

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