Sulfur Cycling-Related Biogeochemical Processes of Arsenic Mobilization in the Western Hetao Basin, China: Evidence from Multiple Isotope Approaches

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DOI: 10.1021/acs.est.6b03460
Published: October 31, 2016
Accepted: October 23, 2016
Received: July 9, 2016

ABSTRACT: The role of sulfur cycling in arsenic behavior under reducing conditions is not well-understood in previous investigations. This study provides observations of sulfur and oxygen isotope fractionation in sulfate and evaluation of sulfur cycling-related biogeochemical processes controlling dissolved arsenic groundwater concentrations using multiple isotope approaches. As a typical basin hosting high arsenic groundwater, the western Hetao basin was selected as the study area. Results showed that, along the groundwater flow paths, groundwater δ34SSO4, δ34OSO4, and δ18OSO4 increased with increases in arsenic, dissolved iron, hydrogen sulfide and ammonium concentrations, while δ13CDOC decreased with decreasing Eh and sulfate/chloride. Bacterial sulfate reduction (BSR) was responsible for many of these observed changes. The δ34SSO4 indicated that dissolved sulfate was mainly sourced from oxidative weathering of sulfides in upgradient alluvial fans. The high oxygen—sulfur isotope fractionation ratio (0.60) may result from both slow sulfate reduction rates and bacterial disproportionation of sulfur intermediates (BDSI). Data indicate that both the sulfate produced by BSR and the overall BDSI reduce arsenic-bearing iron(III) oxhydroxides, leading to the release of arsenic into groundwater. These results suggest that sulfur-related biogeochemical processes are important in mobilizing arsenic in aquifer systems.

INTRODUCTION

High arsenic groundwater has been found worldwide, which has posed health risk to hundreds of millions of people due to its chronic poisoning effects. The role of reducing conditions in the formation, transport, and attenuation of arsenic in reducing groundwater systems is not well understood. The study of the role of sulfur cycling in arsenic behavior under reducing conditions is not well-understood in previous investigations. This study provides observations of sulfur and oxygen isotope fractionation in sulfate and evaluation of sulfur cycling-related biogeochemical processes controlling dissolved arsenic groundwater concentrations using multiple isotope approaches. As a typical basin hosting high arsenic groundwater, the western Hetao basin was selected as the study area. Results showed that, along the groundwater flow paths, groundwater δ34SSO4, δ34OSO4, and δ18OSO4 increased with increases in arsenic, dissolved iron, hydrogen sulfide and ammonium concentrations, while δ13CDOC decreased with decreasing Eh and sulfate/chloride. Bacterial sulfate reduction (BSR) was responsible for many of these observed changes. The δ34SSO4 indicated that dissolved sulfate was mainly sourced from oxidative weathering of sulfides in upgradient alluvial fans. The high oxygen—sulfur isotope fractionation ratio (0.60) may result from both slow sulfate reduction rates and bacterial disproportionation of sulfur intermediates (BDSI). Data indicate that both the sulfate produced by BSR and the overall BDSI reduce arsenic-bearing iron(III) oxhydroxides, leading to the release of arsenic into groundwater. These results suggest that sulfur-related biogeochemical processes are important in mobilizing arsenic in aquifer systems.
aqueous environments. In comparison, large sulfur isotope fractionation has been observed during BSR to sulfide, since sulfate-reducing microorganisms preferentially metabolize isotopically light sulfur (32S)-containing sulfate. Therefore, sulfurs and oxygen isotopes have been used to investigate biogeochemical processes in arsenic-enriched groundwater, showing that BSR occurred in high arsenic groundwater systems. Microbial disproportionation of elemental sulfur was proposed to be coupled with reductive dissolution of iron(III) oxides. However, both the exact sulfur isotope enrichment factor and the ratio of δ34S to δ18O, the important indicators for evaluating sulfur cycling-related biogeochemical processes, are still unclear in high arsenic groundwater systems.

The Hetao basin is a typical basin, hosting high arsenic groundwater (up to 1000 µg/L) with high sulfate concentrations (up to 1200 mg/L). Reductive dissolution of iron(III) oxides has been demonstrated to be an important mechanism for arsenic mobilization based on iron isotope signatures, an incubation study, and hydrogeological and biogeochemical investigation. However, sources of groundwater sulfate and roles of sulfur cycling for arsenic mobility are still unknown, and therefore require further examination. This study reveals sulfur cycling-related biogeochemical processes based on evaluating the sulfur isotope enrichment factor and the ratio of δ34S to δ18O.

The objectives of this paper are to (1) characterize sulfur and oxygen isotopes in sulfate, stable carbon isotopes in dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) in high-arsenic groundwater of the Hetao basin; (2) reveal evaluation of sulfur cycling-related biogeochemical processes controlling dissolved arsenic.

Materials and Methods

The Study Area. The study area includes the alluvial fans and flat plain in the northwest of the Hetao basin with the elevations between 980 and 1050 m above sea level (Figure S1). Alluvial fans, extending from the front of the mountain ranges in the northwest to a broad plain, have topographic gradients between 1/100 and 1/500. The flat plain, comprising around 75% of the study area in the southeast, has a topographic gradient between 1/1000 and 1/5000. In the transition area, the topographic gradient ranges between 1/150 and 1/1000.

Pluvial sediments, typically composed of gravel, coarse sand, and medium sand, mainly occur in the alluvial fans, while fluvial and lacustrine sediments with silt and fine sand were deposited in the flat plain during the Quaternary period. Groundwater mainly occurs in the quaternary alluvial, alluvial–pluvial, and alluvial–lacustrine aquifers. Shallow groundwater is generally hosted in unconfined aquifers overlying the clay layers around 40 m below land surface (bls), while deep groundwater in the confined aquifer normally occurs beneath the clay layers. The depth to the water table generally decreases from around 20 m bls in the alluvial fans to around 2.0 m bls in the flat plain. Details on the study area can be found in the Supporting Information.

Water Sampling and Analysis. Eighty-eight water samples (including 71 groundwater samples, 4 rainwater samples, 4 streamwater samples, 3 samples from an irrigation channel, 4 samples from drainage channels, and 2 lake water samples) were collected. Sampling locations are shown in Figure S1b. Groundwater samples were taken by submersible pumps from public water supply/irrigation wells, including 20 samples from the alluvial fans, 15 samples from the transition area, and 36 samples from the flat plain. The depths of these wells mostly ranged between 40 and 100 m, which commonly extracted deep (confined) groundwater. Details on groundwater sampling and analysis can be found in the Supporting Information.

The δ34S and δ18O were determined using isotope mass spectrometer (Isotope Prime, VG Instruments) in a continuous-flow mode at the Institute of Applied Geosciences, Karlsruhe Institute of Technology (Germany). Prior to analysis of sulfur and oxygen isotope ratios, the dried BaSO4 samples were exchanged to SO4 in an elemental analyzer and CO in a pyrolysis reactor, respectively. The δ34S and δ18O were reported in parts per thousand (‰) deviation from the composition of Vienna Canyon Diablo Troilite (V-CDT) and from Vienna Standard Mean Ocean Water (V-SMOW), respectively. The precisions are better than ±0.2‰ for δ34S and ±0.3‰ for δ18O. δ13C of DOC and δ13C of DIC were determined by Isotope Mass Ratio Spectrometer (MAT-253, Thermo Fisher Scientific), with analytical precisions of 0.17‰ and 0.03‰, respectively.

Sediment Sampling and Analysis. In the flat plain, a representative borehole (BH02) was drilled to take sediment samples in 2012 (Figure S1b). Fifty-three samples were collected at different depths up to 87 m bls. Immediately after being taken out of the borehole, fresh sediments were sealed in N2-filled polyethylene bags, and transported to the laboratory at 4 °C. They were stored frozen at −20 °C in the laboratory. Sediment samples were freeze-dried and disaggregated and then milled for XRD, XRF, and trace element analysis. Details on sediment sampling and analysis can be found in the Supporting Information.

Different arsenic binding forms were sequentially extracted in an anaerobic chamber (Coy Lab, USA) under a N2/9 M HCl, pyrite-S (Cr(II)-reducible arsenic), arsenic coprecipitated with AVS, carbonates, Mn oxides, and very amorphous Fe oxyhydroxides (S3, amorphous monosulfides-coprecipitated arsenic), arsenic incorporated in amorphous iron oxides (S4, amorphous iron oxide-coprecipitated arsenic), arsenic coprecipitated in crystalline iron oxides (S5, crystalline iron oxide-coprecipitated arsenic), arsenic oxides and arsenic coprecipitated with silicates (S6, arsenic oxides), arsenic coprecipitated with pyrite and amorphous As2S3 (S7, pyrite-coprecipitated arsenic), and crystalline arsenic sulfides (S8, arsenic sulfides). Wet samples were used to sequentially extract reduced inorganic sulfur (including dissolved sulfide and FeS, pyrite-S and elemental sulfur) with the method of Hsieh and Shieh. Dissolved sulfide and FeS (AVS) was extracted with deoxygenated 9 M HCl, pyrite-S (Cr(II)-reducible sulfide, CRS) with 2 M CrCl3, and elemental sulfur (ES) with N,N-dimethylformamide (DMF).

Five representative bedrock samples were also collected in the mountains of groundwater recharge areas for determination of sulfur isotope composition of sulfides in rock samples. Analytical details can be found in the Supporting Information.

Data Processing. The local meteoric water line (LMWL) in the Hetao basin is shown as eq. 1.

\[ \delta^18H_{H_2O} = 7.19 \delta^{18}O_{H_2O} + 3.27 \]
To identify deviations from the LMWL, $\delta^{18}$O_H2O-offset is defined as
\[ \delta^{18}$O_H2O-offset = \delta^{18}$O_H2O - (\delta^3$H_H2O - 3.27)/7.19 \tag{2} \]

A Rayleigh distillation model was used to explain the relationship between sulfur isotope ratios and sulfate concentrations during BSR, which is shown as eq 3. The model is valid for closed system conditions, for example deep groundwater in confined aquifers in this study.

\[ \delta = \delta_0 + \varepsilon \times \ln f \tag{3} \]

where $\delta$ and $\delta_0$ are $\delta^{34}S$ of initial sulfate and residual sulfate, respectively; $\varepsilon$ is the fraction of remaining sulfate relative to initial sulfate; $e$ is the sulfur isotopic enrichment factor, which is determined by the slope of the linear trend in a $\delta^{34}$S SO4 versus $\ln f$ plot. In the study area, sulfate concentrations were not only affected by BSR but also by evaporation. To account for greater sulfate concentrations due to evaporation, the fractions of sulfate/chloride meq ratio were used to calculate the $f$ values, instead of sulfate concentrations, as shown in eq 4. This substitution is validated by no other Cl sources in deep groundwater of the study area, with no Cl-evaporites observed along the alluvial fans to the flat plain (Table S1). Groundwater generally evolved from oxic and suboxic conditions to anoxic conditions along the flow paths. Eh values of groundwater samples ranged between 20 and 430 mV (median 90 mV), showing decreasing trends from the alluvial fans (median 270 mV) to the flat plain (median 80 mV) (Table S1). Accordingly, increasing trends in concentrations of DOC, ammonium, hydrogen sulfide and iron(II) were observed from the fans to the flat plain (Figure S3). Groundwaters in the flat plain also had higher dissolved iron and manganese concentrations, ranging between 0.41 and 4.55 mg/L and between 69.3 and 701 μg/L, respectively, in comparison with those in the alluvial fans.

Rainwater and streamwater samples had relatively low TDS (Table S1), with bicarbonate and sulfate as the major anions and calcium and sodium the major cations. Water from irrigation channels had TDS between 547 and 646 mg/L (median 628 mg/L). Because of evaporation and groundwater discharge, water samples from both drainage channels (drainage water) and lakes had high TDS values (medians 1320 and 1260 mg/L, respectively).

Groundwater showed an increasing trend in arsenic concentrations along the flow paths. In general, arsenic concentrations lay in the range between 1.1 and 553 μg/L (median 135 μg/L), with 77% samples exceeding the WHO drinking water guideline of arsenic. In the alluvial fans with Eh values between 60 and 430 mV, groundwater had relatively low arsenic concentrations with a median of 3.0 μg/L. In the transition area, arsenic concentrations ranged from 19.3 to 227 μg/L with a median of 42.5 μg/L. Among 15 studied samples, seven had arsenic concentrations >50 μg/L, which is the drinking water guideline for arsenic in rural areas in China. In the flat plain with Eh values between 20 and 140 mV, groundwater had arsenic concentrations between 18.9 and 553 μg/L (median 239 μg/L). More than 94% of investigated groundwaters contained arsenic >50 μg/L. Arsenic(III) was the major arsenic species, with organic arsenic species (mono- and dimethylarsenic, MMA and DMA) below detection limits (<2.0 μg/L). Arsenic(III) was low in the alluvial fans and high in the flat plain.

**RESULTS**

**Groundwater Chemistry and Arsenic.** Groundwater generally occurred in neutral to weakly alkaline conditions, with pH between 7.2 and 8.5 (median 7.8), generally increasing from the alluvial fans to the flat plain (Table S1). Groundwater total dissolved solid (TDS) ranged between 455 and 5340 mg/L, with calcium and sulfate or bicarbonate in the alluvial fans and sodium and chloride and/or bicarbonate in the flat plain as the predominant cations and anions (Figure S2). Groundwater generally evolved from oxic and suboxic conditions to anoxic conditions along the flow paths. Eh values of groundwater samples ranged between 20 and 430 mV (median 90 mV), showing decreasing trends from the alluvial fans (median 270 mV) to the flat plain (median 80 mV) (Table S1). Accordingly, increasing trends in concentrations of DOC, ammonium, hydrogen sulfide and iron(II) were observed from the fans to the flat plain (Figure S3). Groundwaters in the flat plain also had higher dissolved iron and manganese concentrations, ranging between 0.41 and 4.55 mg/L and between 69.3 and 701 μg/L, respectively, in comparison with those in the alluvial fans.

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**Water Isotopes and Stable Carbon Isotopes.** Groundwater had $\delta^{18}$O and $\delta^2$H between −11.3‰ and −8.7‰ and between −87.4‰ and −66.5‰, respectively (Table S1). All samples are located around or slightly deviate to the right of the LMWL (Figure S4), which indicated the meteoric origin and the effects of evaporation during infiltration and recharge.

Groundwater $\delta^{13}$CDOC ranged between −19.8‰ and −6.2‰ with a median of −11.6‰, which shows a decreasing trend from the alluvial fans (median −8.7‰) to the flat plain (median −12.9‰) (Figure 1, Figure S5). Surface waters generally had constant $\delta^{13}$CDOC between −6.0‰ and −5.0‰ (Table S1), showing the contribution of atmospheric CO2.

![Figure 1. Box-whisker plot of groundwater $\delta^{18}$SSO4, $\delta^{18}$OSO4, $\delta^{13}$CDOC and $\delta^{13}$CDOC from the alluvial fans, the transition area, and the flat plain.](image-url)

In comparison with $\delta^{13}$CDOC, $\delta^{13}$CDOC are lower in water samples (Table S1). The medians of groundwater $\delta^{13}$CDOC (around −25.0‰) were similar for the alluvial fans, the transition area and the flat plain (Figure 1). Relatively low $\delta^{13}$CDOC (−26.0‰) were found in groundwaters near the lakes and the main drainage channel of the flat plain. Surface water samples showed a variation in $\delta^{13}$CDOC between −28.7‰ and −23.9‰ (Table S1).

**Sulfur and Oxygen Isotopes of Dissolved Sulfate.** Large variations in $\delta^{18}$SSO4 (0.5‰ to 27.5‰) and $\delta^{18}$OSO4 (0.8‰ to 13.2‰) of groundwaters were observed, generally showing increasing trends from the alluvial fans to the flat plain (Figures 1 and 2a). In the alluvial fans, groundwater normally had low $\delta^{18}$SSO4 (median 6.7‰) and $\delta^{18}$OSO4 (median 3.3‰), with slightly higher values in the southwest than in the
northeast. In the transition area, $\delta^{34}$SO$_4^-$ and $\delta^{18}$OSO$_4^-$ ranged between 6.4‰ and 14.9‰ (median 10.7‰) and between 2.2‰ and 13.2‰ (median 7.6‰), respectively. In the flat plain with anoxic conditions, groundwaters had the highest $\delta^{34}$SO$_4^-$ and $\delta^{18}$OSO$_4^-$ with medians of 16.2‰, 11.1‰, respectively (Table S1).

The $\delta^{34}$SO$_4^-$ in streamwater samples varied between 0.1‰ and 10.3‰, depending on the sampling location. In the southwest, relatively higher $\delta^{34}$SO$_4^-$ (mean 10.3‰) were observed compared to those in the northeast (mean 0.28‰). However, $\delta^{18}$OSO$_4^-$ of stream waters had a small range between 3.7‰ and 5.3‰. Relative to stream waters, irrigation waters had higher $\delta^{34}$SO$_4^-$ and $\delta^{18}$OSO$_4^-$, with medians of 8.5‰ to 8.9‰ and 10.3‰ to 10.6‰, respectively, which were identical to $\delta^{34}$SO$_4^-$ and $\delta^{18}$OSO$_4^-$ of lake waters. Among surface water samples, drainage waters possessed the highest $\delta^{34}$SO$_4^-$.

**Sediment Geochemistry.** Silty and fine sand samples were mainly composed of quartz, feldspar, muscovite, and carbonates, while silty clay and clay samples mainly contained clay minerals (data not shown). Pyrite (1.2%) was only detected in one sample with high total S contents (2090 mg/kg), which was deep-gray fine sand with organic-enriched clay. No iron oxide minerals were detected due to their low contents (<1%) or poor crystallinity.

Total arsenic contents ranged between 4.44 and 28.9 mg/kg with an average of 12.8 mg/kg (median 10.2 mg/kg), which were well-correlated with Fe contents ($R = 0.88$, $p < 0.01$) and Mn contents ($R = 0.87$, $p < 0.01$) (Figure S6). Sequential extraction of sediment arsenic showed that S2-strongly adsorbed arsenic and S4-amorphous iron oxide-coprecipitated arsenic were the major arsenic forms (Figure 3b and 3c), which averagely accounted for 35% and 23% of totals, respectively (Figure S7b). S1-loosely adsorbed arsenic ranged between <0.05 and 1.11 mg/kg (median 0.38 mg/kg), which is much lower than S2-strongly adsorbed arsenic (median 2.13 mg/kg). Among coprecipitated arsenic, S4-amorphous iron oxide-coprecipitated arsenic was generally the highest (0.52 to 5.57 mg/kg, median 1.51 mg/kg), followed by S3-amorphous...
monosulfides-coprecipitated arsenic, S5-crystalline iron oxide-coprecipitated arsenic, S7-pyrite-coprecipitated arsenic, S6-arсенic oxides, and S8-arсенic sulfides. Aquifer silty sand and fine sand, close to clay layers, generally had high contents of S1-loosely adsorbed arsenic, S2-strongly adsorbed arsenic, S3-amorphous monosulfides-coprecipitated arsenic, especially at depths around 82 m bsl (Figure S7c). Among amorphous monosulfides, amorphous iron oxides and crystalline iron oxides fractions, iron contents in amorphous monosulfide fraction were generally the highest, most around 50% (Figure S7c).

Total sulfur contents ranged between 161 and 2090 mg/kg (median 414 mg/kg), which were poorly correlated with total arsenic contents (R = 0.28, p < 0.01). Sequential extraction of reduced inorganic sulfur showed that CRS (median 66.0 mg/kg) was the major reduced inorganic sulfur form, followed by AVS (median 23.5 mg/kg) and ES (median 2.56 mg/kg) (Figure 3d). Elemental sulfur was generally high (>30 mg/kg) in fine sand neighboring silty clay/clay. A good correlation between S7-pyrite-coprecipitated arsenic and CRS was observed with R = 0.87 (p < 0.01) (Figure S8a). But AVS was poorly correlated with S3, amorphous monosulfides-coprecipitated arsenic (R = 0.06, p < 0.01) (Figure S8b).

**DISCUSSION**

**Sources of Groundwater Sulfate.** Sulfide minerals occur widely in the bedrocks of the mountains, including pyrite, pyrrhotite, chalcopyrite, sphalerite, and galena. Analysis of several sulfide mineral-containing samples (n = 5), taken in the mountains, showed that δ34S ranged between 0.7‰ and 15.7‰, which are heavier than most sulfide minerals normally having δ34S between −50‰ and +10‰.4,21 Little sulfur isotope fractionation occurs during oxidation of sulfide minerals, and thus sulfide-derived sulfate largely reflects the δ34S of the host-rock sulfides.21,59 Groundwaters in the alluvial fans and stream waters had δ34S of sulfide minerals (Figure 2a), indicating that host-rock sulfides were the sources of groundwater sulfate.

The relationship between the positive δ34S of H2O-effect and chloride concentration (Figure S9) indicated that groundwater was affected by evaporation prior to or during recharge. To assess the role of dissolution of evaporites, sulfate concentrations were normalized to chloride concentrations to exclude evaporation effects on the increase in sulfate concentration.58 The δ34S of SO4 relative to sulfate/chloride meq ratios, as shown in Figure 2b, indicated two groups. Groundwaters in Group 1 showed relatively high sulfate/chloride meq ratios (mostly between 1.34 and 5.24) and low δ34S (between 0.54‰ and 7.00‰), which were derived from oxidation of sulfides relatively depleted in 34S. Samples in Group 1 are mostly located in the alluvial fans. One sample in the right of Group 1 had a relatively higher sulfate/chloride ratios and moderate δ34S (5.14‰), which would be affected by dissolution of evaporites with relatively higher δ34S (normally >15‰). Therefore, the contribution of evaporites to groundwater sulfate was less important. Groundwaters in Group 2 were characterized by relatively lower sulfate/chloride ratios but higher δ34S, which are mainly located in the flat plain.

**Isotope Evidence for Bacterial Sulfate Reduction (BSR).** Although groundwaters had a range of initial sulfate concentrations in the alluvial fans, a significant negative correlation between δ34S and sulfate/chloride (R = −0.60; p < 0.01) was observed mainly in the flat plain (Figure 2b). Since bacteria preferentially use 32S sulfate, the remaining sulfate is enriched in 34S as sulfate concentration progressively decreases. This correlation provided strong evidence for BSR occurring in high arsenic groundwater systems.

The initial sulfate/chloride was assigned as 5.23 to calculate the f values (eq 4) in a typical sample taken in the alluvial fans, which was not affected by BSR. As shown in Figure 4, the ε for groundwaters in the transition area and the flat plain was −9.3‰ ± 0.06‰. The value is close to those of other alluvial aquifers (−9.7‰, −9.8‰, and −9.8‰),51,60 however less negative in comparison with leachate-affected alluvial aquifers (−23‰),51 coastal aquifers (−12.3‰),39 and detrital-carbonate aquifers (−20‰).61 The less negative ε value (−9.3‰) obtained in this study may reflect different electron donors and/or different sulfate-reducing organisms.62–65

Bacterial sulfate reduction was also supported by the presence of available sulfate, sulfate reducing bacteria, organic matter, and redox conditions. Initial sulfate concentrations between 145 and 1340 mg/L were sufficient to support BSR in sedimentary aquifers.66 Indigenous sulfate reducing bacteria were found in both groundwaters and aquifer sediments43,67 in the Hetao basin. Reducing conditions were established in the flat plain aquifers with Eh between 20 and 140 mV.

Anaerobic oxidation of organic matter was coupled with BRS in the groundwater. The positive correlation between δ34C of DIC and 1/DIC was observed in the studied groundwater (Figure S5). Because of preferential usage of 13C-depleted organic matter by microbes,70,71 this correlation reflected the contribution of organic matter biodegradation to dissolved inorganic carbon.72 Moreover, lower δ34C of DIC corresponded to lower sulfate/chloride in the flat plain (Figure S10), indicating the role of organic matter biodegradation in BSR.73,74

**Causes of Sulfate—O Fractionation.** Figure 2a shows that ε18O/ε34S approached 0.60. This value is identical to that observed by Aharon and Hu75 and Hosono and others, but relatively higher than those (around 0.25) observed in Canadian River alluvium,51 and the Framvaren Fjord marine basin.76 Generally, BRS consists of four major reversible intracellular steps: sulfate enters the cell (step 1); the sulfate is transformed to adenosine phosphosulfate (APS) via adenosine triphosphate

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**Figure 4.** Plot of δ34S of SO4 versus f (SO4/Cl) in logarithmic scale in groundwaters from the alluvial fans, the transition area and the flat plain. (Legend is the same as in Figure 2.)
(ATP) (step 2); the APS is reduced to sulfite (step 3); and the sulfite is transformed to sulfide (step 4). Step 4 may take place in one step with the enzyme sulfite reductase or through the multiple-step trithionate pathway. Both $^{34}\text{S}$ and $^{18}\text{O}$ of sulfate increase as BRS progresses because most of enzymatic steps preferentially select the lighter sulfur ($^{32}\text{S}$) and oxygen ($^{16}\text{O}$) isotopes. In addition to kinetic oxygen isotope fractionation, $^{18}\text{O}$ exhibits equilibrium oxygen isotope fractionation during BSR, where oxygen isotopes of reduced sulfur intermediates (such as APS and sulfite) exchange oxygen atoms with water. Sulfite in the adenosine monophosphate (AMP)–sulfite complex likely facilitates this oxygen isotope exchange. In the flat plain of the study area, the $^{18}\text{O}$SO$_4$ generally approached a constant (14‰), while the $^{34}\text{S}$SO$_4$ continued to increase (Figure 2a). The difference between $^{34}\text{S}$SO$_4$ and $^{18}\text{O}$SO$_4$ ($^{18}\text{O}$SO$_4$–$^{34}\text{S}$SO$_4$) allows the assessment of kinetic and equilibrium isotope fractionation of sulfate-oxygen during progressive BSR. The relationship between $^{18}\text{O}$SO$_4$ and $^{34}\text{S}$SO$_4$ shown in Figure 5a illustrates that $^{18}\text{O}$SO$_4$ approached an equilibrium value of $24\%$ when $^{34}\text{S}$SO$_4$ were greater than $18\%$.

Two possible reasons can be used to explain the relatively high oxygen–sulfur isotope fractionation ratio ($^{18}\text{O}/^{34}\text{S}$ around 0.60). One is the slow rate of BSR in groundwater systems. The slower rate of BSR would lead to the higher ratio of $^{18}\text{O}$ to $^{34}\text{S}$. Although the slower rate of BSR would increase both $^{34}\text{S}$ and $^{18}\text{O}$, it would be linked to the higher rate of intracellular sulfoxidation, which causes the more rapid increase in the $^{18}\text{O}$SO$_4$ relative to the $^{34}\text{S}$SO$_4$. In groundwater systems, low bioavailability of organic matter would contribute to the slow sulfate reduction rate. A net sulfate reduction rate of $9 \times 10^{-13}$ mol/(L·yr) corresponded to the $^{18}\text{O}/^{34}\text{S}$ ratio of 1.4 in the deep marine sediments, while a higher reduction rate ($3 \times 10^{-5}$ mol/(L·yr)) to the ratio of 0.35 in the shallow estuary sediments, although the ratio-rate relation is still unknown in alluvial sediments. The other reason is bacterial disproportionation of sulfur intermediates (BDSI), as shown in eq 5. Bacterial disproportionation contributed to higher $^{18}\text{O}/^{34}\text{S}$ ratios (up to 0.8), with $^{18}\text{O}$ enrichment in produced sulfate. Contents of elemental sulfur, one of the reactants in eq 5, ranged between 0.15 and 65.6 mg/kg, mostly higher than 6.0 mg/kg at depths between 68 and 81.2 m bsl (Figure 3d). These data support the hypothesis of BDSI in aquifers of the flat plain.

$$4\text{S}^0 + 3\text{Fe}^{2+} + 4\text{H}_2\text{O} \leftrightarrow 3\text{FeS} + \text{SO}_4^{2-} + 8\text{H}^+$$  \hspace{1cm} (5)

**Biogeochemical Processes for Arsenic Enrichment.**

Extensive BSR has resulted in high arsenic concentrations in groundwater. From the alluvial fans to the flat plain, both dissolved arsenic and $^{34}\text{S}$SO$_4$ generally increase, with a positive correlation between them ($R = 0.78$, $p < 0.01$) (Figure 5b). This result indicates that BSR plays an important role in arsenic mobilization, which is consistent with other reports. In this study, BSR led to hydrogen sulfide concentrations of up to 30 µg/L in the flat plain. The presence of hydrogen sulfide leads to reduction of arsenic-adsorbing iron(III) oxyhydroxides as shown in eq 6. In the present study, strongly adsorbed arsenic and amorphous iron(III) oxyhydroxides-coprecipitated arsenic were relatively high (Figure 5b and 5c). This reduction mobilizes the adsorbed or coprecipitated arsenic into groundwater, and therefore increases dissolved arsenic concentrations. The presence of elemental sulfur in aquifer sediments (Figure 3d) confirmed the occurrence of eq 6.

$$3\text{S}^0 + 2\text{FeOOH} \leftrightarrow 2\text{FeS} + \text{SO}_4^{2-} + 2\text{H}^+$$  \hspace{1cm} (7)

Combination of eqs 5 and 6 yields the overall BDSI, eq 7 for disproportionation of $\text{S}^0$ in the presence of iron(III) oxyhydroxides. Anaerobic bacterial disproportionation resulted in enrichment of $^{18}\text{O}$-sulfate, further promoting reductive dissolution of iron(III) oxyhydroxides. The difference between $^{34}\text{S}$SO$_4$ and $^{18}\text{O}$SO$_4$ ($^{18}\text{O}$SO$_4$–$^{34}\text{S}$SO$_4$) was up to 24‰ (Figure 5c), which is identical to those observed in coastal aquifers of southwestern Taiwan (15.1‰ to 24.8‰) and South Korea (5‰ to 26‰), showing anaerobic disproportionation in the presence of iron oxides. The overall BDSI-related reduction of iron(III) oxyhydroxides would also increase groundwater arsenic concentrations.

Sulfide precipitation had a limited role in removing dissolved As from groundwater in this study, although arsenic would be associated with sulfide minerals. Arsenic contents in the AVS-extracted fraction were mostly lower than 0.6 mg/kg in sediments, indicating that limited As was bound to FeS in the studied aquifer sediments. The poor correlation between S3-amorphous monosulfides-coprecipitated arsenic and AVS, as

![Figure 5](image-url)
shown in Figure S8b, also supported this observation. Although there is high affinity for arsenic to coprecipitate in pyrite, contents of S7-pyrite-incorporated arsenic in aquifer sediments were generally low with a median of 0.65 mg/kg (Figure 3) and high arsenic concentrations in groundwater oversaturated with respect to pyrite were normally observed, suggesting that arsenic scavenging by pyrite may be limited. In comparison with S7-pyrite-incorporated arsenic, much lower contents of S8-arsenic sulfoarsenicate (including realgar and orpiment) were observed (averagely 0.14 mg/kg, Figure 3c).

The formation of thioarsenate during BSR also results in arsenic mobility, which was observed in both experiments and in a field site. Although, we detected no thioarsenate species in the studied groundwater samples, possibly due to the alteration of arsenic species and the oxidation of thioarsenate during sampling and sample preservation, the contribution of thioarsenate formation during BSR to arsenic mobility deserves further investigation.

Environmental Implication. Previous studies showed that dissimilatory reduction of iron(III) oxides is the major process for arsenic enrichment in anoxic groundwaters. However, BSR would concomitantly occur with dissimilatory reduction of iron(III) oxides in case of the low solubility of available iron(III) oxides. Although we can not rule out the contribution of biological reduction of iron(III) oxides to arsenic mobility, this study indicates that reduction of iron(III) oxyhydroxides being related to BSR and the overall BDSI contributed to arsenic release into groundwater (Figure 6), which improves our understanding of arsenic mobilization mechanisms.

![Figure 6. Biogeochemical processes for arsenic mobilization in terms of iron–sulfur–arsenic cycling in groundwater systems.](image)

ARSENIC RELEASE AND CAPTURE PROCESSES

BSR leads to the production of sulfide, which may chemically reduce iron(III) oxyhydroxides and subsequently release the adsorbed arsenic into groundwater (Figure 6). This process would also be effective in the Datong high arsenic groundwater system, where both dissolved sulfide and iron(III) oxyhydroxides were present.

In addition to iron(III) oxyhydroxides, elemental sulfur or other sulfur intermediates are the reactants during the overall BDSI, which would occur near the interface of clay layer and fine sand layer in the aquifers. Near the clay layer with high contents of organic matter, BSR was expected to occur and produce dissolved sulfide, which would be transported and/or diffused to fine sand/clay layers with iron(III) oxyhydroxide. Elemental sulfur, produced in the chemical reaction between dissolved sulfide and iron(III) oxyhydroxide, would trigger BDSI as both electron donor and acceptor. A coupling of BSR, sulfide oxidation and BDSI may be the reason that no dissolved sulfide was accumulated in groundwater (mostly <30 μg/L in this study). Bacterial community and dissolved sulfide concentration would be the important factors affecting BDSI. Desulfocapsa thiozymogenes, Desulfobulbus propionicus, and Desulfoacetica cad626 isolated from freshwater systems were reported to thrive on the disproportionation of elemental sulfur. Specific BDSI bacteria in the aquifer sediments need to be characterized to support the occurrence of BDSI in the future study. Although high sulfide concentrations would limit sulfur disproportionation, BSR would not result in high dissolved sulfide due to the form of FeS or pyrite over long-term periods (Figure 6). Therefore, the overall BDSI may occur in heterogeneous aquifers in the presence of relatively low dissolved sulfide (10–30 μg/L) and moderate dissolved sulfate concentrations (around 200 mg/L) under sulfate-reducing conditions.

Dissimilatory reduction of iron(III) oxides also contributed to arsenic release (Figure 6), which would proceed concurrently with BSR when the available iron(III) oxides had a low solubility. In this case, dissimilatory reduction of iron(III) oxides and BSR- and the overall BDSI-induced reduction of iron(III) oxyhydroxides would simultaneously lead to arsenic mobilization. However, at this stage, it is impossible to reveal the relative importance of biological iron(III) oxide reduction to BSR- and the overall BDSI-related iron(III) oxide reduction because of the mutual consumption of organic matter and complex interaction between iron and sulfur.

Further studies are required to quantify their contributions to iron(III) oxyhydroxide reduction as a function of iron(III) oxide mineralogy, microbes, and arsenic species.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03460. Detailed descriptions of the study area, water sampling and analysis, sediment sampling and analysis, and additional figures and tables (PDF)

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Notes
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ACKNOWLEDGMENTS

The study has been financially supported by National Natural Science Foundation of China (Nos. 41672225 and 41222020), the program of China Geology Survey (No. 1212011303700), the Fundamental Research Funds for the Central Universities (No. 2652013028), and the Fok Ying-Tung Education Foundation, China (Grant No. 131017). Furthermore, the authors thank Gesine Prues from KIT Institute of Applied Geosciences for support during isotope ratio measurements. Courtesy review by Dr. Richard Wanty (USGS) is specially acknowledged.

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