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Influence of Sulfate Reduction on Arsenic Migration and Transformation in Groundwater Environment

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Abstract: The sulfate-reducing bacteria-mediated reduction process is considered to be an important mechanism affecting arsenic migration and transformation in anaerobic environments. To investigate the effect of sulfate-reducing bacteria in a high-arsenic aquifer on arsenic migration and transformation, the typical sulfate-reducing bacteria *Desulfovibrio vulgaris* was selected for micro-cosmic experiments to simulate a groundwater environment with or without sulfate amendment. The reduction of Fe(III) and As(V) by *Desulfovibrio vulgaris* was identified, and Fe(III) and As(V) were reduced in both sulfate-free and sulfate-containing systems. However, the addition of 1 mM sulfate significantly enhanced Fe(III) and As(V) reduction. Compared with no sulfate addition, 1 mM sulfate increased the reduction rates of Fe(III) and As(V) by 111.9% and 402.2%, respectively. The sulfate process mediated by *Desulfovibrio vulgaris* also remarkably promoted arsenic release in sediments. These results indicated that sulfate concentration should be considered when sulfate reduction is used as a remediation method for arsenic pollution in groundwater.

Keywords: sulfate reduction; bio-reduction of As/Fe; high arsenic sediments; sulfate concentration

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1. Introduction

Arsenic (As), a toxic metalloid, is widely present in the natural environment and has strong toxicity and carcinogenicity [1]. Endemic arsenic poisoning caused by drinking high-arsenic groundwater is a worldwide geological, environmental, and health problem [2,3]. Long-term drinking of high-arsenic groundwater can cause high-risk diseases such as skin cancer and lung cancer [4,5]. Arsenic and arsenic compounds have mutation, teratogenic, and even carcinogenic properties, and have been listed by the International Agency for Research on Cancer (IARC) as a Class I carcinogen [6]. As the global problem of high arsenic-groundwater becomes more prominent, people are paying more and more attention to the geochemical behavior of arsenic in groundwater. The reduction and dissolution of Fe (hydr) oxide and reduction of arsenate (As(V)) to a more mobile arsenite (As(III)) by microorganisms was considered to be the main reason for the arsenic release into groundwater [7,8].

Sulfate-reducing bacteria (SRB) is a kind of ubiquitous functional bacteria in the environment, which is a strictly anaerobic bacteria capable of sulfate reduction [9,10]. They use oxidized forms of sulfur (such as S_0, S_2O_3, and SO_4^{2-}) as electron acceptors, and sulfides are the final product [11]. Sulfate isfirst reduced to sulfite, and the generated sulfite was then converted to sulfide under the action of sulfite reductase in dissimilatory sulfate pathway [11]. The sulfur cycle is an important part of the geochemical cycle and further affects arsenic cycle [12,13]. Some SRB strains, such as *Desulfovibrio vulgaris* (ATCC strain 7757), can reduce As(V) to As(III) through biological sulfide at high pH [14,15].

Luo et al. [16] confirmed that *Desulfovibrio vulgaris* DP4 mediated the reduction of adsorbed on nano-TiO_2 As(V) by in situ ATR-FITR experiments. However, As(V) reduction was not observed in the abiotic experiment with sulfide as a reducing agent, even if the ratio of sulfide to As(V) reached 524. Dissolved sulfide can serve as an electron donor for abiotic As (V) reduction, but this process is highly dependent on pH [14,17,18]. The molar ratio of sulfide to As(V) was only 2:1, and As(V) reduced to As(III) at pH 4~5. However, even if the molar ratio of the initial sulfide to As(V) reached 100:1 at pH 7, As(V) reduction was not observed [18]. Some SRB strains can directly use As(V) as the electron acceptor for dissimilation arsenic reduction [19,20]. Macy et al. [20] isolated two sulfate-reducing bacteria capable of reducing As(V). When lactic acid was used as an electron donor, the isolated two desulfurization bacteria strains both reduced As(V) and sulfate simultaneously.

More and more evidence has indicated that SRB plays an important role in the biogeochemical cycle of heavy metals in soil [21–23]. The sulfate reduction process generates sulfide under anaerobic conditions, which has an important influence on the migration and transformation of arsenic. Biological sulfide is an effective reducing agent for Fe(III) oxide minerals, which drives arsenic release into groundwater. In addition, sulfide can reduce arsenic migration by promoting the formation of iron sulfide or arsenic sulfide precipitation [24–26]. A previous study has shown that microbial sulfate reduction process in the anaerobic soils of flood zones achieved arsenic sequestration, which was due to As_2S_3 analogue formation related to the Mackinaw mine [27]. The kinetic study of arsenic in Jianghan Plain aquifers demonstrated that microbial-mediated sulfate reduction promoted non-biological reduction of arsenic-containing iron oxides after the artificial input of sulfate [28]. In addition, the presence of thioarsenic species has been found in the groundwater of Bangladesh and Datong Basin, China, in recent years [29,30]. Excessively reduced sulfide reacts with arsenic to form thioarsenic compounds, which enhance arsenic mobility in groundwater [26]. The SRB-mediated reduction process is considered to be a critical mechanism affecting arsenic activation, which is essentially related to the biogeochemical cycle of S [21,31]. However, the explanatory factors that control As mobility are still poorly understood in the SRB-mediated As/Fe/S coupled biogeochemical cycle.

This study aims to explore the effect of SRB action on arsenic migration and transformation by simulating the anaerobic environment of groundwater and utilizing typical SRB *Desulfovibrio vulgaris*. The main aims of this study are to: (i) explore the effect of the bacteria-mediated sulfate reduction process on the biological reduction of Fe(III) and As(V); (ii) determine the effect of the bacteria-mediated sulfate reduction process on arsenic migration and transformation in sediments. The results will be helpful for improving arsenic biogeochemical cycle theory in high-arsenic shallow groundwater systems and provide theoretical guidance for bioremediation of arsenic-contaminated aquifer.

2. Materials and Methods

2.1. Biological Material

A culture of *Desulfovibrio vulgaris* (CGMCC No.:1.5190) was purchased from CGMCC (China General Microbiological Culture Collection Center). *Desulfovibrio vulgaris* was grown to the late exponential phase under anaerobic conditions in Postgate C medium at 37 °C.

2.2. Sample Collection and Characterization

The sediment samples used in the experiments were collected from a depth interval of 18.6–18.8 m from a 50 m deep borehole in Shanyin County (39°21′ N, 112°51′ E) in Datong Basin, Shanxi Province. Core samples packed in polyethylene bags were sealed in a PVC pipe immediately after retrieval, and then transported to the laboratory for freeze-drying. The drying sediments were passed through a 0.149 mm sieve and stored at 4 °C in the dark prior to further experiments.

Physico-chemical parameters of the sediment are summarized in Table 1. Four grams of sample and 10 mL of deionized water were mixed to take pH measurements. The dry sediment (0.5 g) was digested with 9 mL HNO_3 and 3 mL HCl. The concentrations of dis-

solved ions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). All samples were diluted several times to adjust for the operating range and then determined.

Table 1. Physico-chemical parameters of the sediments.

Original Sediment	pH	As mg/kg	Ca g/kg	Mg g/kg	Mn mg/kg	Al g/kg	Fe g/kg
	8.47	18.4	2.71	0.818	48.1	1.64	1.81

2.3. Effects of Sulfate Reduction Process on Dissolved Fe(III) and As(V) Reduction

The reduction effect of *Desulfovibrio vulgaris* on dissolved Fe(III) and As(V) was determined. The artificial groundwater medium with the following constituents was used for the incubation treatments (g L^{-1}): KH_2PO_4, 0.10; NH_4CI, 1.0; $CaCl_2{\cdot}6H_2O$, 0.4; $MgCl_2{\cdot}6H_2O$, 0.1; sodium lactate, 2; yeast extract, 0.2. To investigate the role of sulfate in the Fe(III)/As(V) bio-reduction process under anaerobic conditions, an additional 1 mM sulfate was added to the medium. This ingredient was chosen to represent natural groundwater in a sulfate-reducing environment, and sulfate concentration selection depended on the sulfate content in the sulfate soil wetland groundwater [32–34]. An additional 1 mM ferric citrate or 25 µM arsenate were added to test Fe(III) and As(V) reduction by *Desulfovibrio vulgaris*. There were three parallel treatment groups for each sulfate concentration. The pH of the medium was adjusted to 7.2 with NaOH. Anaerobic bottles were used as a medium for microbial metabolism. The adjusted pH solution was dispensed into 100 mL anaerobic bottles and deoxygenated with pure N_2 for 20 min. The bottles containing culture medium were sealed with thick butyl rubber stoppers (fixed with an aluminum gland) and autoclaved at 121 °C for 30 min. *Desulfovibrio vulgaris* cultured to the late logarithmic stage were washed three times with sterile water in the glove box, and then inserted into anaerobic bottles with 2% inoculum. The anaerobic bottles were placed in an incubator at 32 °C, mixed well every 24 h, and sampled with a syringe after inoculation. All experiments were performed in triplicate. Error bars indicated standard deviation.

2.4. Effects of Sulfate Reduction Process on Migration and Release of Arsenic in Sediments

The composition of the artificial groundwater medium is the same as described in Section 2.2. Sieved sediment samples (20 g) were autoclaved in anaerobic bottles at 121 °C for 30 min, then synthetic groundwater (200 mL) was combined with 0 or 1 mM sulfate. *Desulfovibrio vulgaris* cultured to the late logarithmic stage were washed three times with sterile water in the glove box and then inserted into the anaerobic bottles with 2% inoculum. All the mixtures were cultured in a shaking incubator at 32 °C (200 rpm) and sampled with a syringe after mixing them regularly during the 15-day experiment. All experiments were performed in triplicate and standard deviations were indicated with error bars.

2.5. Sample Analyses

Sub-samples were collected with a sterile syringe for analysis on a regular basis. A 2 mL unfiltered sample was used for pH determination. The cell density (OD_{600nm}) of bacteria was analyzed by UV spectrophotometer (UV-1800PC, Shanghai Mapada Instrument Co., Ltd., Shanghai, China) at λ = 600 nm using 2 mL unfiltered samples. One milliliter of unfiltered sample was mixed with 1 mL of 1 M HCl for 24 h, and then total Fe(II) was quantitated in the filtrate. The sample filtered with a 0.22 µm sterile syringe filter was passed through an ultraviolet spectrophotometer (UV-1800PC, Shanghai Mapada Instruments Co., Ltd., China) to analyze the concentration of dissolved Fe(II), sulfate, and sulfide. Fe(II) concentration was analyzed at 562 nm according to the method used by the authors' research group [35]. Sulfide concentration was determined at 665 nm by methylene blue method [36]. Sulfate concentration was analyzed at 450 nm by Hash SulfaVer 4 sulfate determination reagent powder pillow pack. Samples to be tested for arsenic were pretreated according to Le et al. [37]. Concentrations of arsenic in different species were analyzed

by hydride generationatomic fluorescence spectroscopy (HG-AFS; AFS-830, Beijing Jitian Instrument Co., Ltd., Beijing, China).

Arsenic in sediment samples on the 5th, 10th, and 15th day of the experiment were determined using sequential extraction methods [38]. Briefly, samples were freeze-dried and extracted in the order of eight parts: (F1) ion-bound arsenic; (F2) strongly adsorbed arsenic; (F3) co-precipitated arsenic of minerals sensitive to low pH; (F4) amorphous and weakly crystalline Fe(hydr)oxide bound arsenic; (F5) crystalline iron oxide co-precipitated arsenic; (F6) oxides and silicates co-precipitated arsenic; (F7) pyrite and amorphous As_2S_3 co-precipitated arsenic; (F8) orpiment and remaining recalcitrant arsenic minerals.

3. Results and Discussion

3.1. Effects of Sulfate Reduction Process on Dissolved Fe(III) Reduction

Figure 1 shows the variation of pH value during the Fe(III) reduction experiment. The initial pH was 7.2, and non-biological control groups were basically not changed. The pH of each treatment group decreased after being connected to *Desulfovibrio vulgaris*. The strain utilized carbon source sodium lactate in medium to produce small organic acids and H^+ [39]. Compared with the group without sulfate addition, pH value of the group with 1 mM sulfate was higher due to the alkaline by-product of sulfate reduction [40,41]. The pH of the group treated with 0 and 1 mM sulfate was 6.58 and 6.92, respectively.

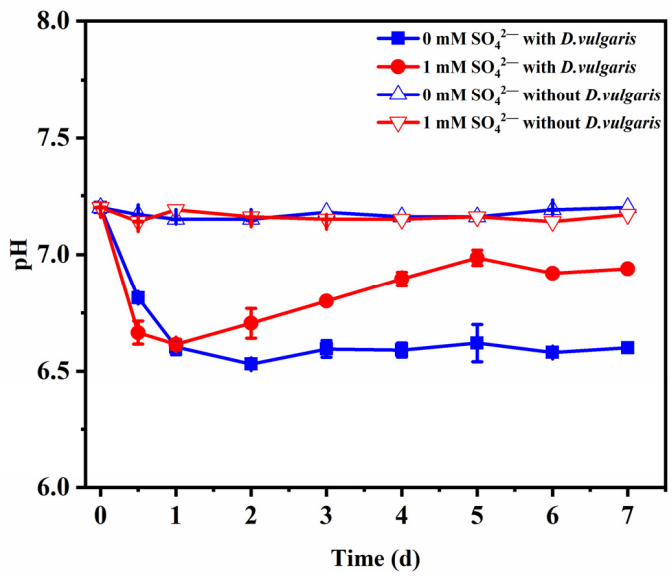

Figure 1. Variation of pH value in Fe(III) reduction experiment. Error bars indicate standard deviation of triplicates.

Figure 2a shows the change of sulfate concentration during the experiment. Sulfate in the 1 mM sulfate treatment group was consumed to the detection limit within 2 days. The reduction of sulfate to sulfide is an eight-electron step process carried out through a variety of intermediates. Sulfate-reducing bacteria usually do not change the intermediate oxidation state and only produce the final product sulfide [39]. Therefore, 1 mM sulfate should have 1 mM sulfide production. However, the detected concentration of sulfide was low, reaching a peak of 105.87 μM on day 2 and decreasing slightly to 99.95 μM at the end (Figure 2b). The result was attributed to the reaction of sulfide with Fe(III) and Fe(II). Sulfide acted as a strong reducing agent of Fe(III) when sulfate was reduced by bacteria; the resulting free sulfide could rapidly reduce Fe(III) (Equation (1)) [42]. In addition, excess sulfide would combine with Fe(II) to form ferrous sulfide (FeS) (Equation (2)) [27,43,44]. The consumption of sulfide made the final detected sulfide concentration in solution much lower than the theoretical value of 1 mM. Figure 2c,d show the changes of dissolved Fe(II) and total Fe(II). The concentration of dissolved Fe(II) and total Fe(II) remained basically

the same in the treatment without sulfate addition. Fe(II) concentration increased to 0.42 mM on the second day and remained stable during the remaining time. The results indicated that *Desulfovibrio vulgaris* had a certain Fe(III) reduction ability, and the reduction rate of Fe(III) was 42%. The production rate of total Fe(II) increased significantly in the sulfate-supplemented treatment group and reached 0.89 mM on day 1, remaining stable thereafter. The increase in Fe(III) reduction rate was due to the abiotic reduction of Fe(III) by sulfide [45].Previous study has shown that Fe(II) concentrations first increased and then decreased in the presence of both microbial-mediated sulfate and Fe(III) reductions [46]. The decrease dissolved Fe(II) concentration was observed within 1–2 days, which was attributed to the reaction of sulfide with Fe(II) to form FeS (Equation (2)) [47]. In addition, the medium without sulfate was brown-green, while the medium with 1 mM sulfate was black at the end of the experiment (FeS; Equation (2)).

$$H_2S + 2Fe^{3+} \rightarrow 2Fe^{2+} + S^0 + 2H^+ \quad (1)$$

$$Fe^{2+} + S^{2-} \rightarrow FeS \downarrow \quad (2)$$

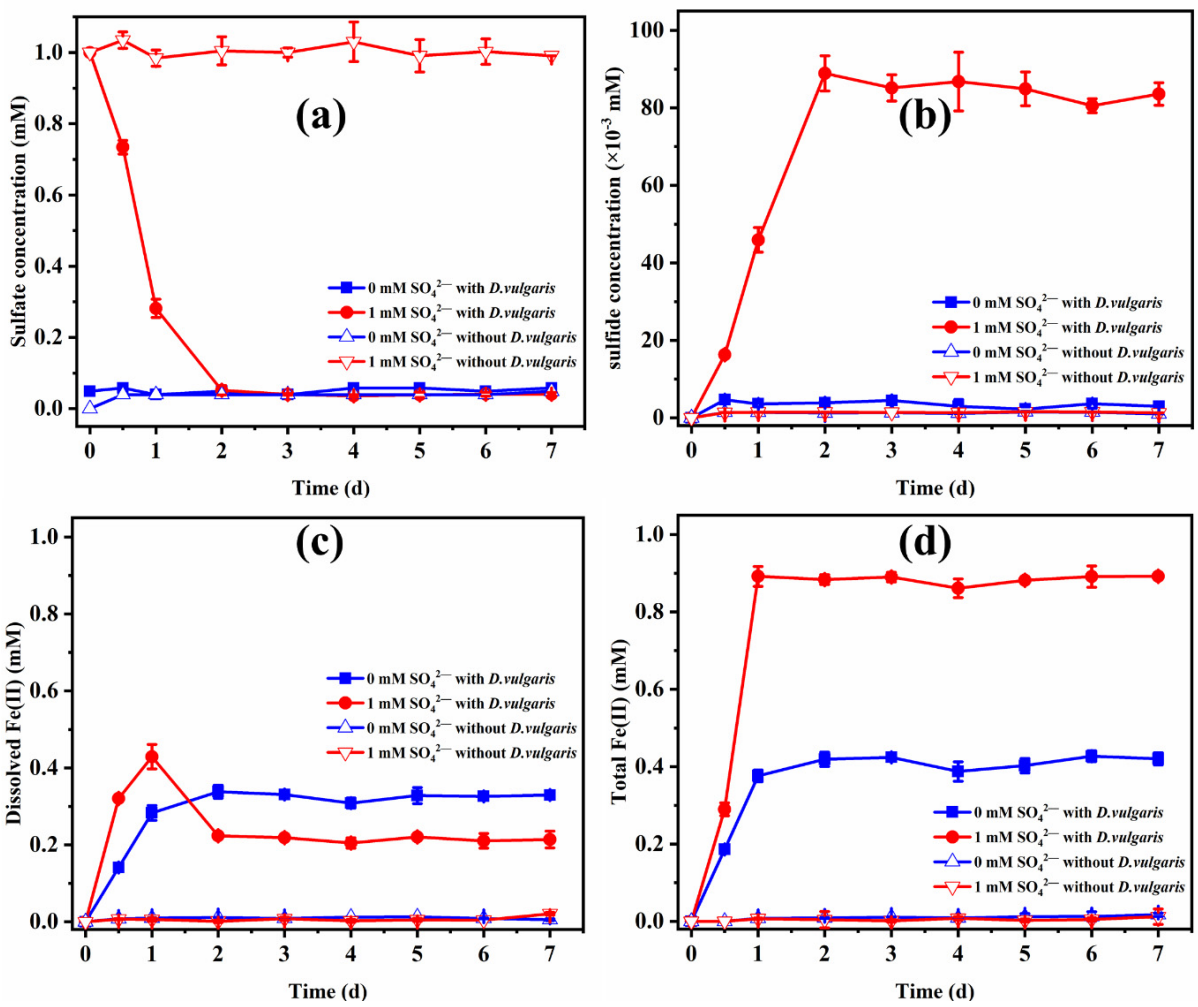

Figure 2. The changes of sulfate (**a**), sulfide (**b**), dissolved Fe (II) (**c**), and total Fe (II) (**d**) concentrations during Fe(III) reduction experiment. Error bars indicate standard deviation of triplicates.

3.2. Effects of Sulfate Reduction Process on Dissolved As(V) Reduction

Figure 3 shows the change of OD_{600nm} of *Desulfovibrio vulgaris*. during the As(V) reduction experiment. The growth of bacteria in the two treatment groups was different. In the treatment without adding sulfate, the OD_{600nm} gradually increased within 0.5 d, then

slowly increased until it reached the maximum value of 0.047 on day 1. There were slight fluctuations in the subsequent incubation time. In the treatment group added with sulfate, the bacteria showed logarithmic growth on day 1, until the OD_{600nm} reached the maximum value of 0.097, which remained stable during the subsequent experimental period. The addition of 1 mM sulfate increased the growth of bacteria by 106.4%. Sulfate-reducing bacteria provide energy for cell synthesis and growth by coupling organic matter oxidation and sulfate reduction [11,39].

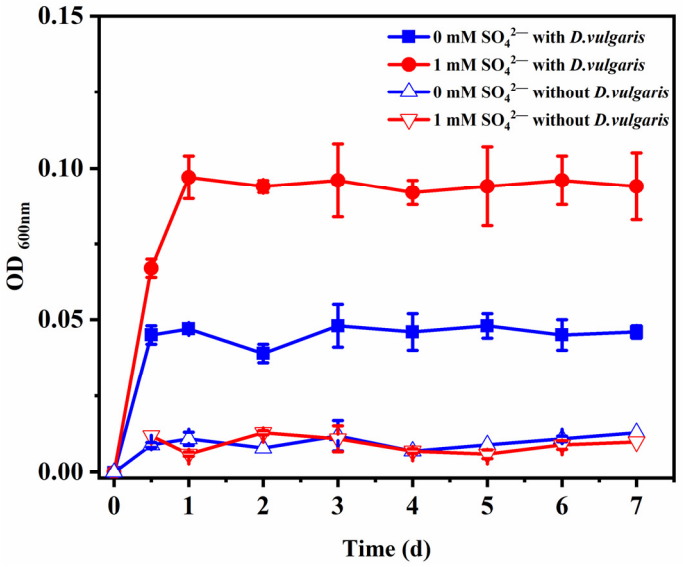

Figure 3. Variation of OD_{600nm} in As(V) reduction experiment. Error bars indicate standard deviation of triplicates.

Figure 4 shows the changes in pH during the experiment. In the treatment group without sulfate, pH value continued to decrease on the first day until it reached 6.84, and then remained basically unchanged. In the treatment group with 1 mM sulfate, the pH value decreased to 7.03 within 0.5 d, then rose to 7.14 on day 1. There was basically no change in the later period of incubation. The pH of the two treatment groups dropped to a certain extent after the strain was connected, which was due to the carbon source sodium lactate being oxidized to release H^+. However, in the treatment group with sulfate addition, the sulfate reduction rate was lower within 0~0.5 d (Figure 5a), mainly due to the oxidation of sodium lactate to produce acid, which lowered the pH value. The sulfate reduction rate was faster within 0.5~1 d (Figure 5a), and the by-products produced in the sulfate reduction process were alkaline, causing a certain increase in pH [40,41]. Sulfate was all reduced after 1 d, and pH value no longer changed.

After inoculation with *Desulfovibrio vulgaris*, sulfate concentration began to decrease with the accumulation of sulfide (Figure 5). Sulfate concentration had been lower than the detection limit after 1 d, and sulfide concentration had increased to 0.98 mM simultaneously. Sulfide concentration decreased slightly to 0.93 mM at the end of the incubation period. It revealed that *Desulfovibrio vulgaris* induced sulfate reduction and sulfide production, and 70 μM sulfide participated in other reactions related to arsenic.

The concentration of As(III), As(V), and total dissolved arsenic [As(T)] in the culture medium was detected (Figure 6). Rochette et al. [20] found that the reduction of As(V) by sulfide was a process strongly related to pH. As(V) reduction was not observed, even when the molar ratio of the initial sulfide to As(V) was 100:1 at pH 7. Due to the neutral pH sulfate reduction system, As(III) may combine with sulfide to form arsenic sulfide, or further form soluble thioarsenite [48–50]. Therefore, As(V) concentration change was used to measure the biological reduction of As(V). As(V) reduction was not observed in non-biological controls. This indicated that the reduction of As(V) was mediated by *Desulfovibrio vulgaris*.

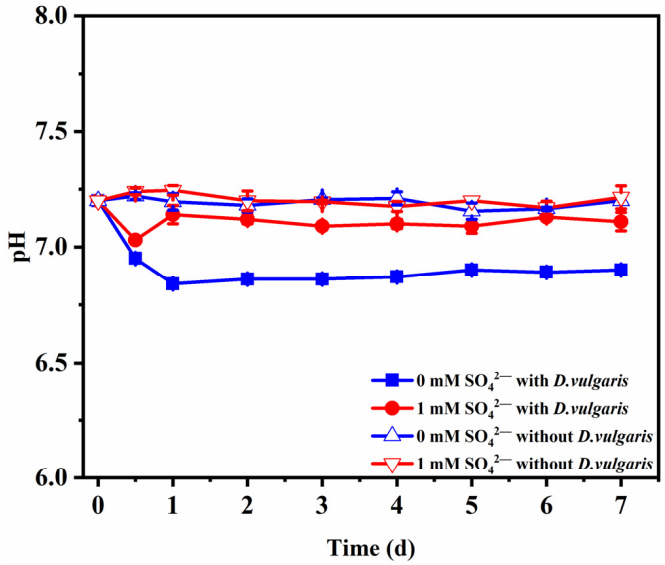

Figure 4. Variation of pH value in As(V) reduction experiment. Error bars indicate standard deviation of triplicates.

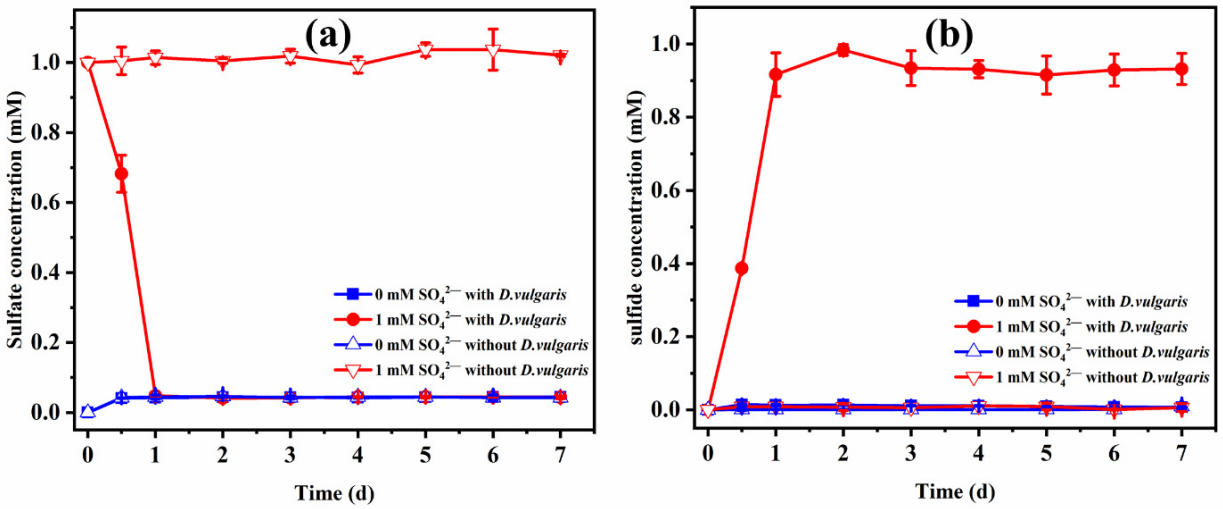

Figure 5. The changes of sulfate (**a**) and sulfide (**b**) concentrations during As(V) reduction experiment. Error bars indicate standard deviation of triplicates.

In the treatment group without adding sulfate, As(V) concentration continued to decrease slowly, and the final concentration was 21.40 μM. The reduction rate of As(V) was 14.4%. Correspondingly, As(III) concentration slowly increased during the experiment until it reached a final concentration of 3.48 μM. As(T) concentration remained constant during the experiment. The results revealed that *Desulfovibrio vulgaris* has a certain As(V) reduction ability. The addition of 1 mM sulfate significantly promoted the reduction of As(V). In the 1 mM sulfate treatment group, As(V) was continuously reduced during the experiment. As(V) concentration dropped to 6.92 μM on day 7, and the As(V) reduction rate reached 72.3%. The addition of sulfate to promote the reduction of As(V) can be explained from two aspects. On the one hand, the addition of 1 mM sulfate significantly promoted the growth of bacteria (Figure 3), which enhanced the reduction of As(V) by the strain. On the other hand, the production of biogenic sulfide promoted the bio-reduction of As(V) [15,51,52]. The concentration of As(III) continued to rise, eventually reaching 7.98 μM. The increase in As(III) concentration was not synchronized with the decrease in As(V) concentration. As(III) can easily combine with free sulfide to form insoluble arsenic-sulfur compounds, such as

orpiment (As_2S_3) (Equation (3)) [17,27]. The decrease in As(T) concentration from 25 μM to 20.03 μM also proved that 19.9% of arsenic was precipitated during the experiment. At the end of the experiment, the difference between the As(T) concentration and the sum of the concentrations of As(III) and As(V) was 5.13 μM, indicating the presence of other soluble arsenic species in the solution in addition to As(III) and As(V). In the sulfate reduction system, this is usually the thioarsenic species [53]. The formation of soluble thioarsenic species was due to the further reaction of excess sulfide with arsenic sulfide precipitation (Equation (4)) [48–50]. These results demonstrated that approximately 25.6% of arsenic existed in the form of thioarsenic at the end of the experiment. Previous study has shown that under the condition of strong sulfate reduction, thioarsenic accounts for a large proportion of total arsenic through thermodynamic simulations. This may be due to the formation of high concentrations of sulfide that promote the formation of thioarsenate [24].

$$H_3As^{III}O_3 + \frac{3}{2}H_2S \rightarrow \frac{1}{2}As_2S_3 + 3H_2O \quad (3)$$

$$As_2S_3 + HS^- + OH^- \rightarrow 2As(OH)S_2^{2-} + H_2O \quad (4)$$

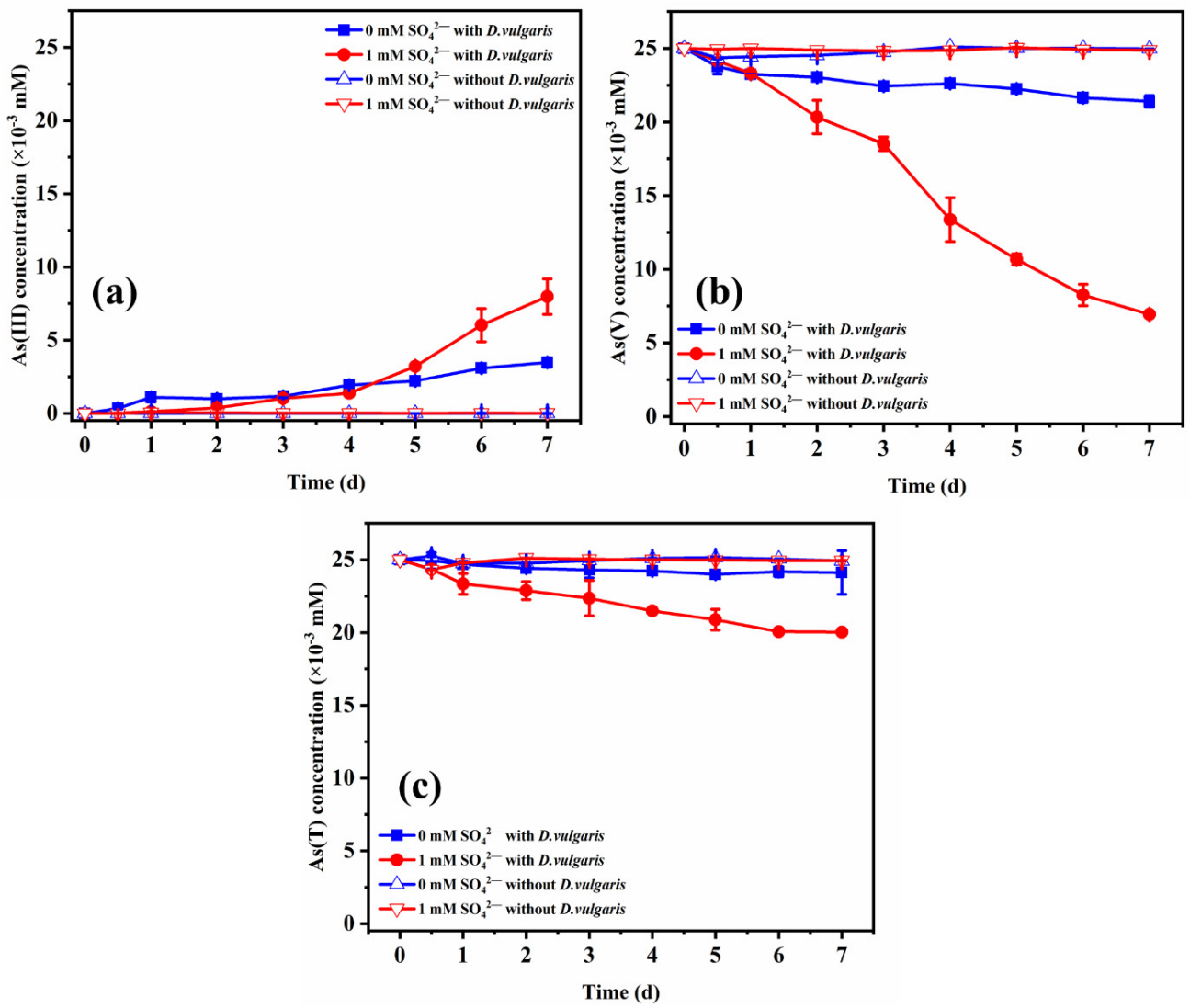

Figure 6. The changes of As(III) (**a**), As(V) (**b**), and As(T) (**c**) concentrations during As(V) reduction experiment. Error bars indicate standard deviation of triplicates.

3.3. Effects of Sulfate Reduction Process on Migration and Release of Arsenic in Sediments

In the biological treatment group, the pH value of both groups first increased and then decreased (Figure 7). The initial increase in pH was due to the higher pH of the soil sample, which increased in a short period of time after mixing with the culture medium. In the treatment group without sulfate, pH value increased within 0~3 d, reached a peak of 7.87 on day 3, and then slowly decreased to 7.61. In the treatment group with 1 mM sulfate added, the pH value rose to 7.93 within 2 days, and then slowly decreased to 7.63. The reduction rate of 1 mM sulfate was the fastest in 1~2 d (Figure 8a), and the by-products in the reduction process were alkaline, so pH reached the maximum on day 2. The concentration of 1 mM sulfate was lower than the detection limit on day 4, indicating that sulfate in the medium was basically consumed. Sulfide reached a maximum of 82.37 μM on the second day, and then dropped to a very low concentration. The results suggested that sulfide produced by sulfate reduction participated in reactions in the sediment system.

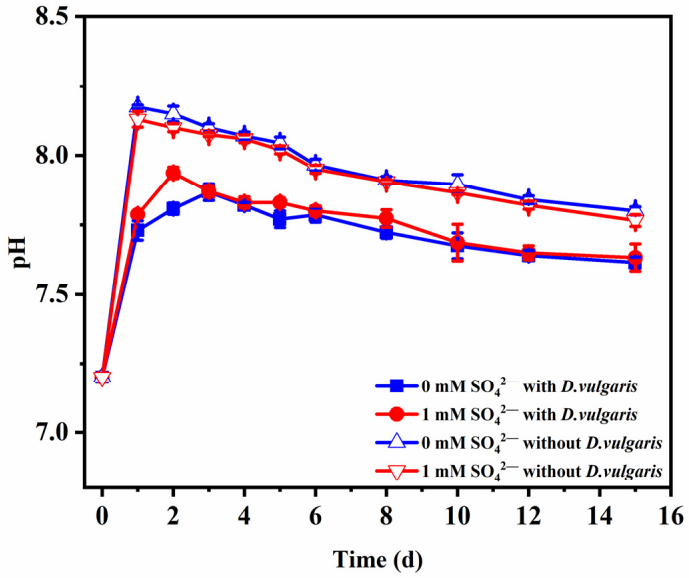

Figure 7. Variation of pH value in sediment experiment. Error bars indicate standard deviation of triplicates.

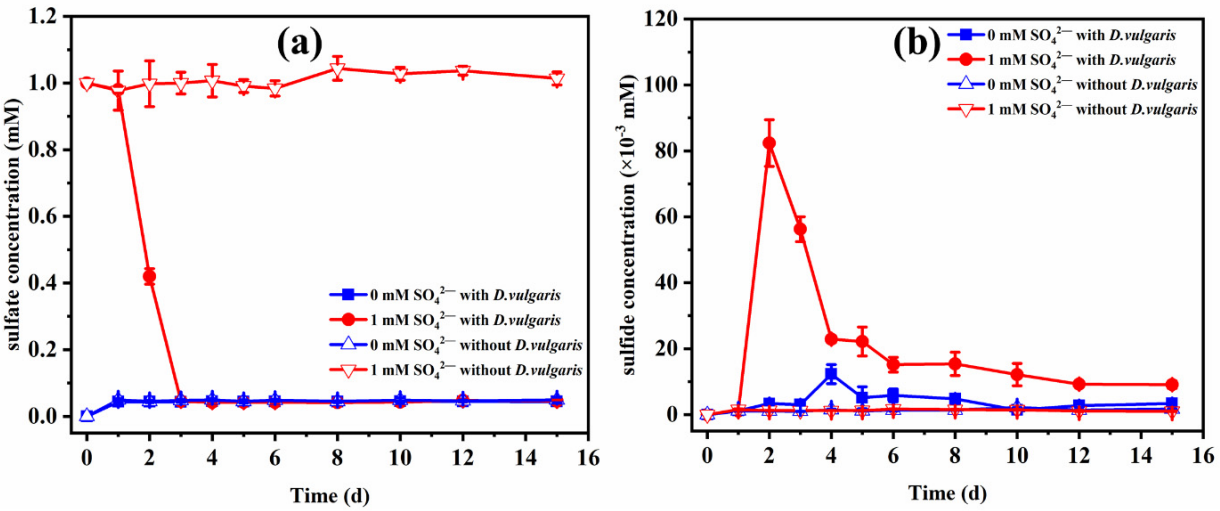

Figure 8. The changes of sulfate (**a**) and sulfide (**b**) concentrations during sediment experiment. Error bars indicate standard deviation of triplicates.

The changes of dissolved As(III), As(V), and As(T) during the sediment experiment were measured (Figure 9a–c). In the abiotic controls, the concentrations of As(III), As(V), and As(T) were maintained at a low level, and the release of Fe(II) was not detected. The result shows that in the absence of bacterial action, the sediment is stable. After bacteria inoculation in the group without sulfate, As(V) and As(T) were released into the water phase in the first two days, and reached the peak values of 0.50 μM and 0.63 μM, respectively on day 2, which were basically constant during the subsequent cultivation period. As(III) concentration remained at a low level throughout the experiment. Corresponding to the liquid phase experiment, *Desulfovibrio vulgaris* had a weaker ability to reduce As(V) without adding sulfate. Sulfate significantly promoted arsenic release in the sediments. In the group added with 1 mM sulfate, As(V) and As(T) were released rapidly in the first 4 days, and reached the maximum value of 1.05 and 2.20 μM on day 4. The concentration of As(III) also reached 0.97 μM. Sulfate reduction was completed within 4 days simultaneously (Figure 8), which revealed that sulfate reduction was beneficial to the release of arsenic into the water phase.

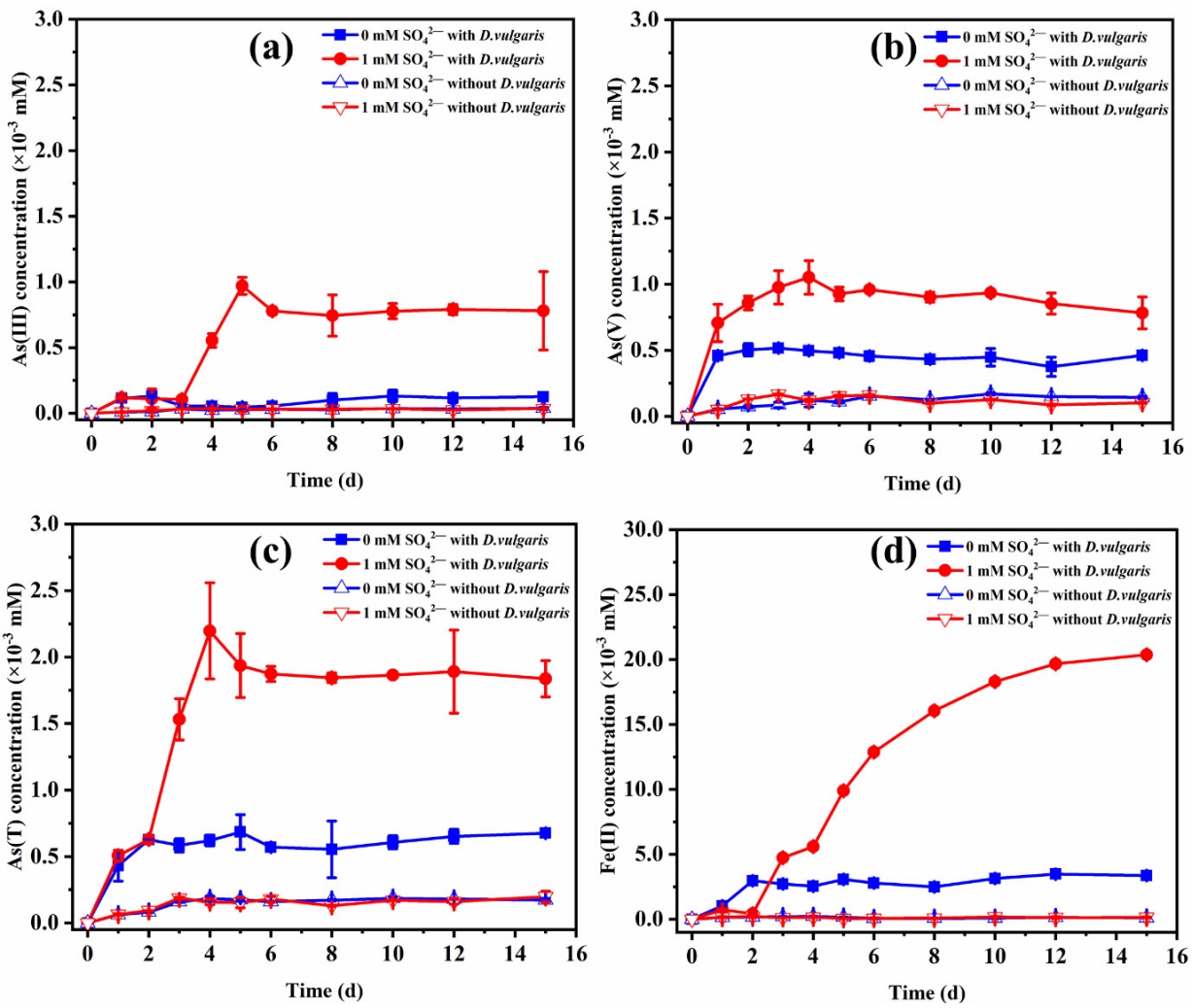

Figure 9. The changes of As(III) (**a**), As(V) (**b**), As(T) (**c**), and Fe(II) (**d**) concentrations during sediment experiment. Error bars indicate standard deviation of triplicates.

Arsenic released into groundwater was mainly due to the microbially mediated reduction of As(V) to As(III) and reductive dissolution of arsenic-loaded Fe (hydr) oxides [7,54]. According to the reduction experiment of dissolved Fe(III) and As(V) by *Desulfovibrio vulgaris*, the sulfate reduction process significantly promoted Fe(III) and As(V) reduction

(Figures 2 and 6). Therefore, the sulfate reduction process mediated by the strain can significantly promote arsenic release in the sediments. Specifically, in the early stage of the experiment, the observed reduction of As(V) was relatively weak. It suggested that As(III) desorption by Fe(hydr)oxides was not the main process of arsenic mobilization. The release of arsenic may be mainly due to the abiotic reduction of arsenic-containing Fe(hydr) oxides in sediments (Equation (5)). At the same time, the concentration of dissolved S^{2-} and Fe^{2+} was at a relatively low level, which corresponded to results of the liquid phase experiment, indicating that sulfide combined with ferrous iron while reducing iron (hydr) oxide. In a previous study, it was found that strong sulfate reduction resulted in high arsenic and low iron levels at the end of the incubation [24]. On the third day of incubation, As(III) concentration in the group treated with 1 mM sulfate was increased. This was due to the reduction of As(V) to As(III) by SRB reduction, resulting in arsenic release. With 1 mM sulfate addition, As(T) concentration decreased slightly after reaching the peak, which was attributed to the resorption of arsenic by FeS. Studies have shown that FeS was the first authigenic mineral formed during the sulfidation of arsenic-containing Fe(hydr) oxides [55]. FeS can adsorb arsenic released into the aqueous solution again, thereby affecting the concentration of arsenic in groundwater and the redistribution of arsenic in sediments [56,57]. Fe^{2+} concentration began to rise after 4 days, indicating that the iron sulfide precipitation reached equilibrium.

$$HS^- + 2Fe(OH)_3(s) + 5H^+ \leftrightarrow 2Fe^{2+} + S^0(s) + 6H_2O \quad (5)$$

Under anaerobic conditions, the relative content of different forms of arsenic in the sediments mediated by *Desulfovibrio vulgaris* is shown in Figure 10. Eight-step extraction of arsenic was performed on the samples on the 5th, 10th, and 15th days. The relative content of F6 in the two treatment groups increased, mainly due to the large change in the water-soluble arsenic content. Because the water-soluble arsenic content in the sediments is relatively small under actual conditions, this situation is not discussed. In the experimental group without sulfate, the strong adsorption of arsenic (F2) and co-precipitated arsenic of minerals sensitive to low pH (F3) decreased from 11.50% and 11.47% to 2.54% and 9.86%, respectively. This suggested that arsenic release was mainly from these two parts, and remaining components had no obvious changes. In the experimental group added with 1 mM sulfate, the proportions of strongly-adsorbed arsenic (F2) and amorphous and weakly crystalline Fe(hydr)oxide bound arsenic (F4) decreased from 11.5% and 9.93% to 5.25% and 8.72%, respectively. It revealed that the sulfate reduction process mediated by *Desulfovibrio vulgaris* promoted arsenic release from F2 and F4 components, and made F2 and F4 components transform to other components. Compared with crystalline iron oxides, bacteria are more likely to reduce iron oxide minerals with poor crystals [58]. In addition, F3 increased from 10.4% to 12.5%, indicating that arsenic in the F3 component increased under sulfate reduction action. The arsenic includes arsenic combined with carbonates and irregular sulfide and oxides [38]. Ferrous sulfide was irregular sulfide, indicating that formed ferrous sulfide may have an adsorption effect on arsenic. This was consistent with the change of As and Fe concentrations (Figure 8). In addition, arsenic in the F5 component increased from 12.7% to 15.4%. This illustrated that arsenic in the remaining components may be converted to arsenic bound to crystalline iron oxide under sulfate reduction, resulting in arsenic readsorption. Arsenic in the F8 fraction increased from 0.173% to 0.199%, which suggested that there were also a small amount of refractory arsenic minerals such as orpiment. This indicates that the sulfate reduction process significantly affects arsenic species transformation in sediments.

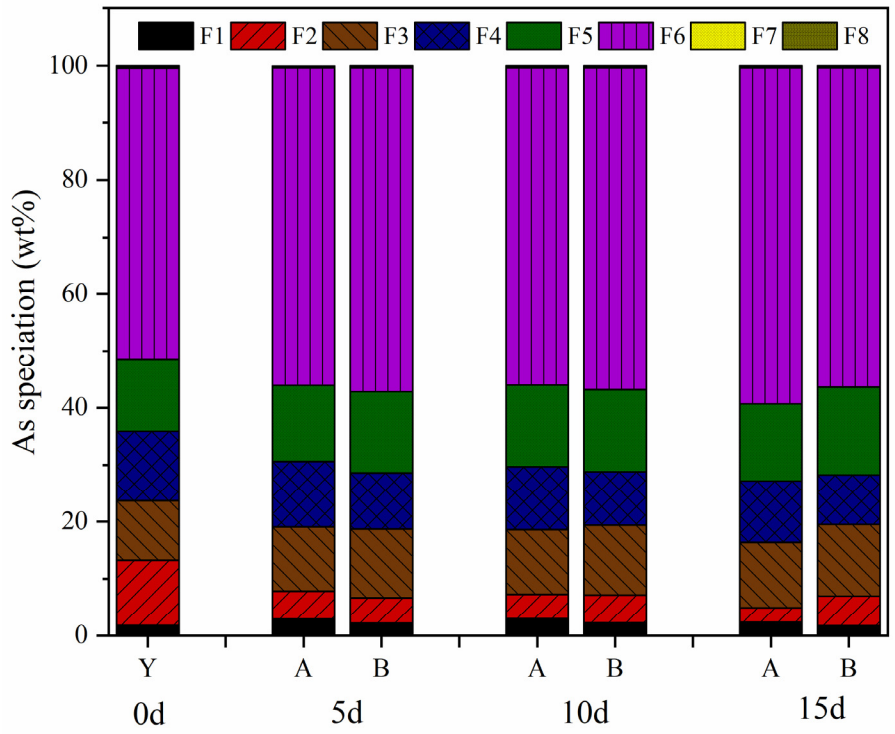

Figure 10. Determination of combined forms of arsenic by Keon continuous extraction method: ion-bound arsenic (F1); strongly adsorbed arsenic (F2); co-precipitated arsenic of minerals sensitive to low pH (F3); amorphous and weakly crystalline Fe(hydr)oxide bound arsenic (F4); crystalline Fe(hydr)oxide co-precipitated arsenic (F5); arsenic co-precipitated with oxides and silicates (F6); arsenic co-precipitated with pyrite and amorphous As_2S_3 (F7); orpiment and remaining recalcitrant arsenic minerals (F8). Y: initial soil; A: Soil + *Desulfovibrio vulgaris* + 0 mM sulfate treatment; B: Soil + *Desulfovibrio vulgaris* + 1 mM sulfate treatment).

4. Conclusions

This study aimed to explore the effect of sulfate reduction mediated by *Desulfovibrio vulgaris* on the reduction and release of As/Fe in a groundwater environment. Microcosm experiments demonstrated that 1 mM sulfate significantly enhanced *Desulfovibrio vulgaris* growth and bio-sulfide formation, and thus stimulated the reduction of dissolved As (V) and Fe (III). Because the main pathways of arsenic migration from sediment to groundwater were the reduction and dissolution of arsenic-bearing iron oxides and the reduction of arsenate to more easily migrated arsenite, sulfate reduction significantly promoted arsenic mobilization in the sediments from these two aspects. The formed ferrous sulfide had a certain adsorption effect on arsenic, which slightly reduced the arsenic concentration in the aqueous phase. However, the release of arsenic at the 1 mM sulfate level was much greater than adsorption. The migration and transformation of arsenic are strongly affected by sulfate reduction. When using biological sulfate reduction as a groundwater remediation strategy, sulfate concentration should be considered.

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References

1. Ramsay, L.; Petersen, M.M.; Hansen, B.; Schullehner, J.; Wens, P.; Voutchkova, D.; Kristiansen, S.M. Drinking water criteria for arsenic in high-income, low-dose countries: The effect of legislation on public health. *Environ. Sci. Technol.* **2021**, *55*, 3483–3493. [CrossRef]
2. Christodoulidou, M.; Charalambous, C.; Aletrari, M.; Kanari, P.N.; Petronda, A.; Ward, N.I. Arsenic concentrations in groundwaters of cyprus. *J. Hydrol.* **2012**, *468–469*, 94–100. [CrossRef]
3. Palansooriya, K.N.; Shaheen, S.M.; Chen, S.S.; Tsang, D.C.; Hashimoto, Y.; Hou, D.; Ok, Y.S. Soil amendments for immobilization of potentially toxic elements in contaminated soils: A critical review. *Environ. Int.* **2020**, *134*, 105046. [CrossRef] [PubMed]
4. Rasool, A.; Farooqi, A.; Masood, S.; Hussain, K. Arsenic in groundwater and its health risk assessment in drinking water of mailsi, punjab, pakistan. *Hum. Ecol. Risk Assess.* **2016**, *22*, 187–202. [CrossRef]
5. Zhou, Y.; Wang, J.; Wei, X.; Ren, S.; Yang, X.; Beiyuan, J.; Zhang, W. Escalating health risk of thallium and arsenic from farmland contamination fueled by cement-making activities: A hidden but significant source. *Sci. Total Environ.* **2021**, *782*, 146603. [CrossRef]
6. Sommella, A.; Deacon, C.; Norton, G.; Pigna, M.; Violante, A.; Meharg, A.A. Total arsenic, inorganic arsenic, and other elements concentrations in italian rice grain varies with origin and type. *Environ. Pollut.* **2013**, *181*, 38–43. [CrossRef] [PubMed]
7. Guo, H.; Liu, Z.; Ding, S.; Hao, C.; Xiu, W.; Hou, W. Arsenate reduction and mobilization in the presence of indigenous aerobic bacteria obtained from high arsenic aquifers of the hetao basin, inner mongolia. *Environ. Pollut.* **2015**, *203*, 50–59. [CrossRef] [PubMed]
8. Ye, L.; Wang, L.Y.; Jing, C.Y. Biotransformation of adsorbed arsenic on iron minerals by coexisting arsenate-reducing and arsenite-oxidizing Bacteria. *Environ. Pollut.* **2020**, *256*, 113471. [CrossRef] [PubMed]
9. Muyzer, G.; Stams, A.J.M. The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* **2008**, *6*, 441–454. [CrossRef]
10. Roy, A.; Sar, P.; Sarkar, J.; Dutta, A.; Kazy, S.K. Petroleum hydrocarbon rich oil refinery sludge of north-east india harbours anaerobic, fermentative, sulfate-reducing, syntrophic and methanogenic microbial populations. *BMC Microbiol.* **2018**, *18*, 151. [CrossRef] [PubMed]
11. Qian, Z.; Tianwei, H.; Mackey, H.R.; van Loosdrecht, M.C.M.; Guanghao, C. Recent advances in dissimilatory sulfate reduction: From metabolic study to application. *Water Res.* **2019**, *150*, 162–181. [CrossRef] [PubMed]
12. Kao, Y.H.; Wang, S.W.; Liu, C.W.; Wang, P.L.; Wang, C.H.; Maji, S.K. Biogeochemical cycling of arsenic in coastal salinized aquifers: Evidence from sulfur isotope study. *Sci. Total Environ.* **2011**, *409*, 4818–4830. [CrossRef] [PubMed]
13. Katrin, H.; Maher, W.A.; Stott, M.B.; Frank, K.; Simon, F.; Moreau, J.W. Microbial contributions to coupled arsenic and sulfur cycling in the acid-sulfide hot spring champagne pool, New Zealand. *Front. Microb.* **2014**, *5*, 569. [CrossRef]
14. Burton, E.D.; Johnston, S.G.; Planer-Friedrich, B. Coupling of arsenic mobility to sulfur transformations during microbial sulfate reduction in the presence and absence of humic acid. *Chem. Geol.* **2013**, *343*, 12–24. [CrossRef]
15. Luo, T.; Ye, L.; Ding, C.; Yan, J.; Jing, C. Reduction of adsorbed As(V) on nano-TiO_2 by sulfate-reducing bacteria. *Sci. Total Environ.* **2017**, *598*, 839–846. [CrossRef] [PubMed]
16. Luo, T.; Tian, H.; Guo, Z.; Zhuang, G.; Jing, C. Fate of arsenate adsorbed on nano-TiO_2 in the presence of sulfate reducing bacteria. *Environ. Sci. Technol.* **2013**, *47*, 10939–10946. [CrossRef] [PubMed]
17. Johnston, S.G.; Burton, E.D.; Keene, A.F.; Planer-Friedrich, B.; Voegelin, A.; Blackford, M.G. Arsenic mobilization and iron transformations during sulfidization of As(V)-bearing jarosite. *Chem. Geol.* **2012**, *334*, 9–24. [CrossRef]
18. Rochette, E.A.; Bostick, B.C.; Li, G.; Fendorf, S. Kinetics of arsenate reduction by dissolved sulfide. *Environ. Sci. Technol.* **2000**, *34*, 4714–4720. [CrossRef]
19. Blum, J.S.; Kulp, T.R.; Han, S.; Lanoil, B.; Saltikov, C.W.; Stolz, J.F. *Desulfohalophilus alkaliarsenatis* gen. nov. sp. nov., an extremely halophilic sulfate- and arsenate-respiring bacterium from Searles lake, California. *Extremophiles* **2012**, *16*, 727–742. [CrossRef] [PubMed]
20. Macy, J.M.; Santini, J.M.; Pauling, B.V.; O'Neill, A.H.; Sly, L.I. Two new arsenate/sulfate-reducing bacteria: Mechanisms of arsenate reduction. *Arch. Microbiol.* **2000**, *173*, 49–57. [CrossRef] [PubMed]
21. Duan, Y.; Gan, Y.; Wang, Y.; Liu, C.; Yu, K.; Deng, Y. Arsenic speciation in aquifer sediment under varying groundwater regime and redox conditions at jianghan plain of central china. *Sci. Total Environ.* **2017**, *607–608*, 992–1000. [CrossRef] [PubMed]
22. Li, X.; Dai, L.; Zhang, C.; Zeng, G.; Liu, Y.; Zhou, C. Enhanced biological stabilization of heavy metals in sediment using immobilized sulfate reducing bacteria beads with inner cohesive nutrient. *J. Hazard. Mater.* **2016**, *324*, 340–347. [CrossRef] [PubMed]

23. Jing, R.; Kjellerup, B.V. Biogeochemical cycling of metals impacting by microbial mobilization and immobilization. *J. Environ. Sci.* **2018**, *66*, 149–157. [CrossRef] [PubMed]
24. Gao, J.; Zheng, T.; Deng, Y.; Jiang, H. Microbially mediated mobilization of arsenic from aquifer sediments under bacterial sulfate reduction. *Sci. Total Environ.* **2021**, *768*, 144709. [CrossRef] [PubMed]
25. Pi, K.; Wang, Y.; Xie, X.; Ma, T.; Su, C.; Liu, Y. Role of sulfur redox cycling on arsenic mobilization in aquifers of Datong Basin, northern China. *Appl. Geochem.* **2016**, *77*, 31–43. [CrossRef]
26. Sun, J.; Quicksall, A.N.; Chillrud, S.N.; Mailloux, B.J.; Bostick, B.C. Arsenic mobilization from sediments in microcosms under sulfate reduction. *Chemosphere* **2016**, *153*, 254–261. [CrossRef] [PubMed]
27. Burton, E.D.; Johnston, S.G.; Peter, K.; Bush, R.T.; Salirian, C. Sulfate availability drives divergent evolution of arsenic speciation during microbially mediated reductive transformation of schwertmannite. *Environ. Sci. Technol.* **2013**, *47*, 2221–2229. [CrossRef]
28. Zheng, T.; Deng, Y.; Wang, Y.; Jiang, H.; Xie, X.; Gan, Y. Microbial sulfate reduction facilitates seasonal variation of arsenic concentration in groundwater of Jianghan Plain, Central China. *Sci. Total Environ.* **2020**, *735*, 139327. [CrossRef] [PubMed]
29. Planer-Friedrich, B.; Schaller, J.; Wismeth, F. Monothioarsenate occurrence in bangladesh groundwater and its removal by ferrous and zero-valent iron technologies. *Environ. Sci. Technol.* **2018**, *52*, 5931–5939. [CrossRef] [PubMed]
30. Sun, S.; Xie, X.; Li, J.; Qian, K.; Chi, Z. Distribution and formation of thioarsenate in high arsenic groundwater from the Datong Basin, northern china. *J. Hydrol.* **2020**, *590*, 125268. [CrossRef]
31. Shi, W.; Song, W.; Luo, Y.; Qile, G.; He, J. Transformation pathways of arsenic species: Srb mediated mechanism and seasonal patterns chemosphere. *Chemosphere* **2021**, *263*, 128255. [CrossRef] [PubMed]
32. Root, R.A.; Vlassopoulos, D.; Rivera, N.A.; Rafferty, M.T.; Andrews, C.; O'Day, P.A. Speciation and natural attenuation of arsenic and iron in a tidally influenced shallow aquifer. *Geochim. Cosmochim. Acta* **2009**, *73*, 5528–5553. [CrossRef]
33. Johnston, S.G.; Keene, A.F.; Bush, R.T.; Burton, E.D.; Sullivan, L.A.; Isaacson, L.S.; Powell, B. Iron geochemical zonation in a tidally inundated acid sulfate soil wetland. *Chem. Geol.* **2011**, *280*, 257–270. [CrossRef]
34. Burton, E.D.; Johnston, S.G. Impact of silica on the reductive transformation of schwertmannite and the mobilization of arsenic. *Geochim. Cosmochim. Acta* **2012**, *96*, 134–153. [CrossRef]
35. Wang, J.; Xie, Z.; Wang, Y.; Yang, Y.; Chen, M. Synergy between indigenous bacteria and extracellular electron shuttles enhances transformation and mobilization of Fe(III)/As(V). *Sci. Total Environ.* **2021**, *783*, 147002. [CrossRef] [PubMed]
36. Eaton, A.D.; Clesceri, L.S.; Greenberg, A.E.; Franson, M.A.H. Standard methods for the examination of water and wastewater. *Am. J. Public Health Nations Health* **1966**, *56*, 387–388. [CrossRef]
37. Le, X.C.; Yalcin, S.; Ma, M. Speciation of submicrogram per liter levels of arsenic in water: On-site species separation integrated with sample collection. *Environ. Sci. Technol.* **2000**, *34*, 2342–2347. [CrossRef]
38. Keon, N.E.; Swartz, C.H.; Brabander, D.J.; Harvey, C.; Hemond, H.F. Validation of an arsenic sequential extraction method for evaluating mobility in sediments. *Environ. Sci. Technol.* **2001**, *35*, 2778–2784. [CrossRef] [PubMed]
39. Rosenberg, E.; Delong, E.F.; Lory, S.; Stackebrandt, E.; Thompson, F. *Prokaryotic Physiology and Biochemistry*; Springer: Berlin/Heidelberg, Germany, 2013. [CrossRef]
40. Alam, R.; McPhedran, K. Applications of biological sulfate reduction for remediation of arsenic—A review. *Chemosphere* **2019**, *222*, 932–944. [CrossRef]
41. Gao, K.; Guo, C.; Dang, Z. Mobilization of arsenic during reductive dissolution of As(V)-bearing jarosite by a sulfate reducing bacterium. *J. Hazard. Mater.* **2021**, *402*, 123717. [CrossRef]
42. Flynn, T.M.; O'Loughlin, E.J.; Mishra, B.; Dichristina, T.J.; Kemner, K.M. Sulfur-mediated electron shuttling during bacterial iron reduction. *Science* **2014**, *344*, 1039–1042. [CrossRef] [PubMed]
43. Huang, F.G.; Jia, S.Y.; Liu, Y.; Wu, S.H.; Han, X. Reductive dissolution of ferrihydrite with the release of As(V) in the presence of dissolved S(-II). *J. Hazard. Mater.* **2015**, *286*, 291–297. [CrossRef] [PubMed]
44. Xu, L.; Wu, X.; Wang, S.; Yuan, Z.; Xiao, F.; Yang, M. Speciation change and redistribution of arsenic in soil under anaerobic microbial activities. *J. Hazard. Mater.* **2016**, *301*, 538–546. [CrossRef] [PubMed]
45. Knappová, M.; Drahota, P.; Falteisek, L.; Culka, A.; Penížek, V.; Trubač, J.; Mihaljevič, M.; Matoušek, T. Microbial sulfidogenesis of arsenic in naturally contaminated wetland soil. *Geochim. Cosmochim. Acta* **2019**, *267*, 33–50. [CrossRef]
46. Xia, X.; Yang, W.; Huang, Y. Dissimilatory iron and sulfate reduction by native microbial communities using lactate and citrate as carbon sources and electron donors. *Ecotoxicol. Environ. Saf.* **2019**, *174*, 524–531. [CrossRef] [PubMed]
47. Zhang, S.; Jia, S.; Yu, B.; Liu, Y.; Wu, S.; Han, X. Sulfidization of As(V)-containing schwertmannite and its impact on arsenic mobilization. *Chem. Geol.* **2016**, *420*, 270–279. [CrossRef]
48. Bostick, B.C.; Fendorf, S.; Brown, G.E. In situ analysis of thioarsenite complexes in neutral to alkaline arsenic sulphide solutions. *Mineral. Mag.* **2005**, *69*, 781–795. [CrossRef]
49. de Matos, L.P.; Costa, P.F.; Moreira, M.; Gomes, P.C.S.; de Queiroz Silva, S.; Gurgel, L.V.A.; Teixeira, M.C. Simultaneous removal of sulfate and arsenic using immobilized non-traditional SRB mixed culture and alternative low-cost carbon sources. *Chem. Eng. J.* **2018**, *334*, 1630–1641. [CrossRef]
50. Fendorf, S.; Michael, H.A.; van Geen, A. Spatial and temporal variations of groundwater arsenic in south and southeast Asia. *Science* **2010**, *328*, 1123–1127. [CrossRef] [PubMed]
51. Johnston, S.G.; Keene, A.F.; Burton, E.D.; Bush, R.T.; Sullivan, L.A.; McElnea, A.E.; Ahern, C.R.; Smith, C.D.; Powell, B. Arsenic mobilisation in a seawater inundated acid sulfatesoil. *Environ. Sci. Technol.* **2010**, *44*, 1968–1973. [CrossRef] [PubMed]

52. Saalfield, S.L.; Bostick, B.C. Changes in iron, sulfur, and arsenic speciation associated with bacterial sulfate reduction in ferrihydrite-rich systems. *Environ. Sci. Technol.* **2010**, *43*, 8787–8793. [CrossRef] [PubMed]
53. Wilkin, R.T.; Wallschläger, D.; Ford, R.G. Speciation of arsenic in sulfidic waters. *Geochem. Trans.* **2003**, *4*, 1. [CrossRef]
54. Bose, P.; Sharma, A. Role of iron in controlling speciation and mobilization of arsenic in subsurface environment. *Water Res.* **2002**, *36*, 4916–4926. [CrossRef]
55. Wolthers, M.; Charlet, L.; Weijden, C.; Linde, P.; Rickard, D. Arsenic mobility in the ambient sulfidic environment: Sorption of arsenic(V) and arsenic(III) onto disordered mackinawite. *Geochim. Cosmochim. Acta* **2005**, *69*, 3483–3492. [CrossRef]
56. Gonzalez-Silva, B.M.; Briones-Gallardo, R.; Razo-Flores, E.; Celis, L.B. Inhibition of sulfate reduction by iron, cadmium and sulfide in granular sludge. *J. Hazard. Mater.* **2009**, *172*, 400–407. [CrossRef] [PubMed]
57. Postma, D.; Jessen, S.; Hue, N.T.M.; Mai, T.D. Mobilization of arsenic and iron from red river floodplain sediments, vietnam. *Geochim. Cosmochim. Acta* **2010**, *74*, 3367–3381. [CrossRef]
58. Lovley, D.R.; Holmes, D.E.; Nevin, K.P. Dissimilatory Fe(III) and Mn(IV) reduction. *Adv. Microb. Physiol.* **2004**, *49*, 219–286. [CrossRef]