Science of the Total Environment 954 (2024) 176529

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Sulfate-mediated Fe(III) mineral reduction accelerates arsenic mobilization by a *Desulfovibrio* strain isolated from paddy soil

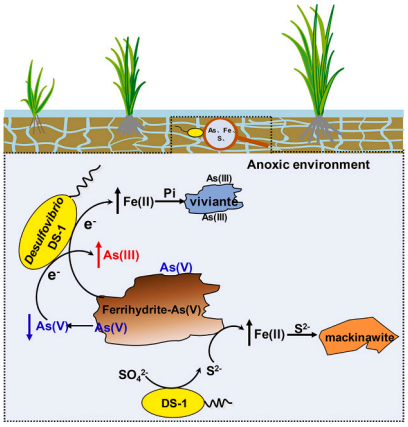
Yi-Fei Wu [a,1], Hui Huang [b,1], Jun Zhang [a,*], Gang Hu [c], Jiajia Wang [d], Chao Peng [e], Andreas Kappler [f], Fang-Jie Zhao [a]

[a] Jiangsu Key Laboratory for Organic Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
[b] College of Ecology and Environment and Co-Innovation Center for the Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, Jiangsu 210037, China
[c] College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China
[d] School of Ecology, Shenzhen Campus of Sun Yat-Sen University, Shenzhen 518107, People's Republic of China
[e] College of Life Sciences, China West Normal University, Nanchong, China
[f] Geomicrobiology, Department of Geoscience, University of Tuebingen, Tuebingen 72076, Germany

HIGHLIGHTS

- *Desulfovibrio* sp. DS-1 shows versatility in reducing Fe(III), As(V), and sulfate
- Strain DS-1 reduces As(V)-bearing ferrihydrite, converting As(V) to As(III)
- Sulfide produced by strain DS-1 enhances ferrihydrite reduction during sulfate reduction
- Sulfate addition exacerbates arsenic mobilization into the aqueous phase

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Xinbin Feng

Keywords:
Arsenate
Mobilization
Ferrihydrite
Sulfate-reducing bacterium

ABSTRACT

The biogeochemical cycling of arsenic (As) is often intertwined with iron (Fe) and sulfur (S) cycles, wherein Fe (III)- and sulfate-reducing bacteria (SRB) play a crucial role. Here, we isolated strain DS-1, a strictly anaerobic Fe (III)- and sulfate-reducing bacterium, from As-contaminated paddy soil. Using 16S rRNA gene sequence analysis, strain DS-1 was identified as a member of the genus *Desulfovibrio*. Strain DS-1 utilized energy derived from ferrihydrite reduction to support its cellular growth. Under anoxic sulfate-reducing conditions, the presence of strain DS-1 significantly increased As mobilization compared to sulfate-free conditions. Mechanistically, SRB-

* Corresponding author.
E-mail address: zhangjun1208@njau.edu.cn (J. Zhang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scitotenv.2024.176529
Received 10 September 2024; Received in revised form 21 September 2024; Accepted 23 September 2024
Available online 27 September 2024

Secondary mineral
Mackinawite

produced sulfide reacts with Fe(III) to form FeS, which disrupts Fe(III) minerals, thereby enhancing As release. These findings highlight the critical role of redox disequilibrium in As mobilization and suggest that SRB-produced sulfide may permeate to the rice rhizosphere, increasing As mobilization through Fe(III) reduction.

1. Introduction

Arsenic (As) is a metalloid that can enter the environment through natural processes and human activities, such as mining activities and the use of As-containing pesticides or herbicides (Li et al., 2011). Arsenic is notorious for its toxic effects, including being carcinogenic, teratogenic, and mutagenic (Tseng et al., 1968). In nature, arsenic exists mainly in two oxidation states: trivalent arsenite (As(III)) and pentavalent arsenate (As(V)). The dominant form depends on the redox conditions of the environment. As(III) is generally more toxic than As(V) (Oremland and Stolz, 2003).

Rice paddies, which are periodically flooded, create anoxic conditions that promote the growth of certain microorganisms such as anaerobic iron (Fe)- and sulfate-reducers, fermentative bacteria, and methanogenic archaea (Kirk, 2004). These microorganisms play a key role in As biotransformation in paddy environments (Chen et al., 2019; Wang et al., 2019; Zhang et al., 2015). Under anoxic flooding conditions, there is typically an increase in the concentration of As(III) in the soil porewater (Takahashi et al., 2004; Xu et al., 2017; Xu et al., 2019). The mobility of As is strongly influenced by its interactions with Fe(III) (oxyhydr)oxides through adsorption and/or coprecipitation (Aeppli et al., 2019). The formation, dissolution, and transformation of Fe(III) (oxyhydr)oxides have a significant impact on the fate and mobility of As in nature (Muehe et al., 2016; Yu et al., 2016). Arsenic shows a significant affinity for Fe(III) (oxyhydr)oxides in soil. On the one hand, As(V), the oxidized form of As, readily interacts with these iron minerals (Dixit and Hering, 2003). On the other hand, As(III) is adsorbed to Fe(III) (oxyhydr)oxides less strongly and is therefore more mobile than As(V). Dissimilatory Fe(III) reduction in anoxic environments has received considerable attention in understanding As behavior (Dai et al., 2020; Islam et al., 2004). Furthermore, As(V)-reducing bacteria have been found to affect the mobility of As in complex ways (Kubeneck et al., 2024). However, more studies are needed to explore the impact of diverse microbial metabolisms on As behavior in suboxic and anoxic environments. To develop a comprehensive understanding of the behavior of As in soils, it is crucial to thoroughly investigate the interactions among As, Fe(III) (oxyhydr)oxides, and various soil components, such as sulfates and phosphates.

The biogeochemical cycles of iron and sulfur are frequently interconnected in nature, and their interactions strongly influence the mobility of As (Burton et al., 2011a; Kumar et al., 2020; Ma et al., 2022; ThomasArrigo et al., 2020; Zhao et al., 2017). Sulfur-metabolizing bacteria play a critical role in these cycles, particularly in the transformation of sulfur and arsenic in the environment (Yin et al., 2022). These bacteria participate in processes such as sulfur oxidation and reduction, which have significant implications for the bioavailability and mobility of As in nature. Microbial sulfate reduction plays a significant role in determining the mobility of As in paddy soils (Fang et al., 2024; Fang et al., 2023; Xu et al., 2019). The reduction of sulfate to sulfide leads to the immobilization of As(III) through the precipitation of minerals like arsenic sulfide and iron sulfide (Gao et al., 2021; He et al., 2019; ThomasArrigo et al., 2020; Wang et al., 2023). Sulfide is a common species in subsurface environments and can be produced locally by sulfate-reducing bacteria (SRB) (Demin et al., 2024). SRB are known to play a crucial role in the cycling of Fe, sulfur, and As in various natural environments. Initially, it was thought that SRB indirectly reduce Fe(III) through sulfide production. However, it has been discovered that some SRB may also directly reduce Fe(III) through enzymatic mechanisms (Lovley, 1993a). Studies have shown that different strains of SRB can reduce poorly crystalline Fe(III) (oxyhydr)oxides in pure cultures (Lovley, 1993b).

As(V) reduction serves both resistance and energy generation through different mechanisms. As(V) resistance involves a small As(V) reductase (e.g., ArsC, Car1) and an As(III)-specific efflux pump (e.g., ArsB, ACR3) (Shen et al., 2024; Silver and Phung, 2005). Dissimilatory As(V) reduction, generating energy via oxidative phosphorylation, uses the respiratory As(V) reductase Arr, a heterodimer with a catalytic subunit (ArrA) containing a molybdenum center and a [4Fe—4S] cluster, and a smaller subunit (ArrB) with at least three [4Fe—4S] clusters (Saltikov and Newman, 2003). The involvement of SRB in the reduction of As(V) is still debated. Previous studies have shown that some SRB can enzymatically reduce As(V) as part of a detoxification process and potentially as an energy-conserving respiratory mechanism (Andres and Bertin, 2016; Li and Krumholz, 2007; Macy et al., 2000). For example, *Desulfomicrobium* sp. Ben-RB, a strictly anaerobic SRB species, has been found to utilize As(V) as an sole electron acceptor for energy production (Macy et al., 2000). However, other studies suggest that As(V) reduction by SRB may primarily serve as a detoxification mechanism (Li and Krumholz, 2007). Yet, the role of As(V) detoxification reduction in soil is still unknown. Previous study has indicated that the *ars* operon in SRB may have a minimal impact on the release of adsorbed As(V) on soil minerals (Langner and Inskeep, 2000). However, other study has suggested that the arsenic-resistant (*ars*) operon can lead to increased mobility of As under anoxic conditions (Li and Krumholz, 2007). It is important to note that these findings were observed in laboratory studies, and their significance in the paddy environment is still not well understood. Further study is needed to understand the mechanisms of microbial-mediated As biotransformation in anoxic environments, particularly focusing on the involvement of SRB in flooded paddy soils.

The main objectives of this study are: (1) to investigate the coupled release and reduction mechanisms of As and Fe by pure sulfate-reducing bacteria using batch experiments. The aim is to understand how microbial processes contribute to the mobilization of As and Fe in the environment; (2) to determine the effect of additional sulfate for the release of As in an As-contaminated paddy soil. This will help to elucidate the role of different factors in influencing the mobility of As in As-contaminated paddy environments.

2. Material and methods

2.1. Soil collection

A soil sample for enrichment cultures was collected from the surface layer of a heavy-metal contaminated paddy soil (25°34′43.25″N, 113°01′23.77″E) in Chenzhou city, Hunan province, China. The soil had an organic matter content of 30.8 g kg^{-1}, pH 6.7 (in water), and a total SO_4^{2-}-S content of 143.9 mg kg^{-1}. The soil exhibited a total As concentration of 85.8 mg kg^{-1}, with 42.9 mg kg^{-1} of As being extractable using ammonium oxalate. Detailed soil properties have been previously documented by Zhang et al. (2017).

2.2. Bacterial strains, growth media, and culture conditions

Anoxic freshwater medium was utilized to enrich sulfate-reducing bacteria from paddy soil. The FWM medium composition per liter of distilled deionized water included: 0.3 g of NH_4Cl, 0.4 g of $MgCl_2$ $6H_2O$, 0.6 g of KH_2PO_4, and 0.1 g of $CaCl_2$ $2H_2O$, 1 mL resazurin. After autoclaving and cooling under an atmosphere of 80 % N_2–20% CO_2 (vol/vol), 30 mL of $NaHCO_3$ solution (1 mol/L, autoclaved under CO_2), vitamins, a nonchelated mixture of trace elements were added. The pH

was adjusted to 7.0. Enrichment cultures were supplied with sulfate (10 mmol/L) as an electron acceptor, 10 mmol/L lactate was supplied as the electron donor and carbon source. Fresh paddy soil samples (5.0 g) were mixed homogeneously with 100 mL of anoxic water, and 5 mL of the suspension was added to serum bottles containing 50 mL of the medium. The bottles were then incubated anoxically at 30 °C in the dark. The presence of SRB species was confirmed through the appearance of a black precipitate of FeS, indicating the reduction of sulfate. After three successive transfers in FWM medium containing lactate and sulfate, agar shake cultures were prepared. Single colonies were then picked up from a diluted sample and transferred to FWM medium with lactate and sulfate. Fully grown cultures derived from these colonies were streaked onto agar plates, resulting in the isolation of several morphologically identical isolates. Among these isolates, strain DS-1, was selected for further characterization. Strain DS-1 has been deposited at the Marine Culture Collection of China (MCCC 1K09141).

For routine cultivation of strain DS-1 in batch experiments, strain DS-1 was anaerobically cultured at 30 °C in FWM medium containing 10 mmol/L lactate and 10 mmol/L sulfate, respectively. Prior to use, late-exponential-phase cultures of strain DS-1 were harvested, washed twice in sterile 0.8 % NaCl solution, and the cell concentration was determined by measuring the optical density (OD) at 600 nm. All anaerobic procedures and sample manipulations were performed within a Coy anaerobic chamber utilizing a gas mixture of N_2, CO_2, and H_2 (90:5:5).

2.3. DNA extraction, PCR amplification, and phylogenetic analysis of strain DS-1

The DNA extraction, genome sequencing, and annotation of strain DS-1 were carried out as previously detailed. The draft genome of strain DS-1 is available in GenBank (GCA_004103805.1). To ensure the accuracy of gene annotation, sequences were verified by blasting against the NCBI (https://www.ncbi.nlm.nih.gov/) and Uniprot (https://www.uniprot.org/) databases. Additionally, partial 16S rRNA genes were amplified using PCR with primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′, *Escherichia coli* positions 8–27) and 1492R (5′-TACCTTGTTACGACTT-3′, *E. coli* positions 1507−1492), followed by sequencing. The resulting 16S rRNA gene sequences were subjected to a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) to determine their sequence identity. Subsequently, a neighbor-joining phylogenetic tree based on the 16S rRNA gene sequence was constructed using MEGA 5.0 software. To validate As(V) reduction and sulfate reduction, the gene clusters responsible for these processes were manually extracted from the draft genome of strain DS-1, and sequences of related taxa were obtained from the GenBank database for comparative analysis. The 16S rRNA gene sequence of strain DS-1 has been deposited in GenBank under the accession number PQ013734.

2.4. Anaerobic Fe(III) and arsenate reduction experiments

i) Fe(III) reduction experiments

To determine the Fe(III) reduction activity, strain DS-1 was cultured in FWM medium supplemented with lactate (10 mmol/L) as the electron donor and ferrihydrite (5 mmol/L) as the electron acceptor. The ferrihydrite was synthesized using $FeCl_3$ and NaOH following the method described by Schwertmann and Cornell (2000). The Fe(III) stock solution was prepared using $FeCl_3{\cdot}6H_2O$ (Sigma-Aldrich). Fe(II) concentrations in the cultures were determined by periodically sampling and analysis using the ferrozine method (Stookey, 1970). A 10 % inoculum was used throughout the experiments, and tubes were incubated horizontally and shaken every day to ensure the uniform distribution of bacteria and ferrihydrite.

ii) Arsenate reduction experiments

For the determination of As biotransformation, a lactate-sulfate medium containing 100 μmol/L As(V) was used. The As(V) stock solution was prepared by dissolving $Na_2HAsO_4{\cdot}7H_2O$ (98 %, Sigma-Aldrich) in deionized water. Arsenic from sterilized stock solutions was spiked into the media. Typically, 1 mL of the culture in the late-exponential growth phase ($OD_{600nm} \approx 1.0$) was inoculated into 19 mL of sterile medium. The concentrations of As(III) and As(V) in the cultures were determined using high-performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICP-MS).

2.5. Microbial reduction of As(V)-adsorbed ferrihydrite

The microbially mediated reductive dissolution and transformation of As(V)-adsorbed ferrihydrite were studied using strain DS-1. A suspension of DS-1 cells (1 mL) ($OD_{600nm} \approx 1.0$) was inoculated into anoxic FWM medium (19 mL), which contained 5 mmol/L of As(V)-adsorbed ferrihydrite as the sole electron acceptor. Ferrihydrite with an initial Fe(III) to As(V) ratio of 100 to 1 (As mol/Fe mol) was investigated, which is approximately equivalent to 75:1 (*w*/w). Additionally, lactate (10 mmol/L) was added to the FWM medium as the electron donor. The concentrations of As(III), As(V), and Fe(II) in the microcosms were analyzed using the methods described elsewhere.

To investigate the role of sulfate for microbial As transformation, sulfate (10 mmol/L) was added to 19 mL of FWM medium containing As (V)-adsorbed ferrihydrite, as described above. The bottles were then sealed tightly and incubated in the dark. At indicated time points, the culture samples were homogenized by shaking and then analyzed.

2.6. Arsenic release from sterile soil inoculated with washed cells of strain DS-1

To gain a detailed understanding of the metabolic versatility of strain DS-1, we investigated the ability of strain DS-1 to release As from the soil. The soil was sterilized by gamma-ray irradiation at a dose of 50 kGy. A soil slurry was prepared by mixing 20 g of dry soil with 40 mL of sterilized distilled water in 100 mL serum bottles. To create anoxic environment, the serum bottles were flushed with a stream of N_2 gas and sealed with butyl rubber stoppers and aluminum caps. Lactate (10 mmol/L) was added to the soil slurry. As a control, a background soil was prepared without the addition of strain DS-1. The soil slurries were incubated in the dark at 30 °C without shaking for a duration of 20 d. After the incubation, As(III), As(V), Fe(II), Mn(II) and sulfate in the soil porewater were analyzed. Additionally, the adsorbed As in the residual soil was extracted using 0.6 mol/L ortho-phosphoric acid and 0.1 M ascorbic acid following the method described by Giral et al. (2010).

3. Analytical methods

Protein concentrations were determined using the bicinchoninic acid (BCA) spectrophotometric method. Ferrous iron was measured photometrically at 562 nm following the ferrozine method as described by Stookey (1970. Before sampling, cultures were agitated to disperse iron precipitates. Anoxic syringes were used to collect samples, which were then immediately acidified through a 10-fold dilution in 1 mol/L HCl. The concentrations of sulfide in the samples were determined using a colorimetric method by methylene blue formation (Cline, 1969). At the end of the experiment, the replicates exhibiting As removal were sampled for solid-phase analysis. Details information regarding the analysis of Fe and As, mineral analysis using transmission electron microscopy (TEM/EDX), as well as X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) can be found in the Supplementary Information (SI).

4. Results

4.1. Enrichment and identification of an anaerobic sulfate-reducing bacterial strain

To confirm the sulfate-respiring capability of strain DS-1, we characterized its growth when SO_4^- served as the sole electron acceptor (Fig. 1a and b). Strain DS-1 exhibits the capability to reduce millimolar quantities of SO_4^- when cultivated with lactate (Fig. 1a). The initially added 10 mmol/L SO_4^- were metabolized down to 3 mmol/L within 48 h and thereafter remained relatively constant. Sulfide (S^{2-}) concentration increased to 4 mmol/L during the first 48 h of incubation and remained stable until 96 h (Fig. 1a). However, under these conditions, the process of sulfate reduction did not align closely with cell growth. The most rapid sulfate reduction was observed between 0 and 48 h, whereas the most substantial cell growth took place between 72 and 120 h (Fig. 1b). Given that significant cell growth occurred after the cessation of sulfate reduction, it remains uncertain which electron acceptor drove the cellular growth beyond 48 h (Fig. 1b). Controls without SO_4^- exhibited neither SO_4^- reduction nor cell growth (Fig. 1a and b). In addition, strain DS-1 was unable to grow under oxic or microoxic conditions, indicating that it is an obligate anaerobe. When strain DS-1 utilizes lactate as an electron donor, it is capable of utilizing sulfate, thiosulfate, or sulfite as an electron donor for growth, but it is unable to grow using elemental sulfur (S_0) as an electron acceptor.

Phylogenetic analyses of the 16S rRNA sequence confirmed that strain DS-1 belongs to the class *Deltaproteobacteria* and the genus *Desulfovibrio*. Analysis of the 16S rRNA gene of strain DS-1 showed a high sequence similarity of 99 % to *D. oxamicus* DSM 1925 (NR043567), which is an anaerobic strain known for its ability to reduce sulfate (Fig. S1). This indicates that strain DS-1 is closely related to *D. oxamicus* and falls within the same taxonomic group.

Analysis of the genome unveiled a cluster of three genes potentially encoding a transcriptional regulator from the ArsR family (*arsR*), an arsenic metallochaperone protein (*arsD*), and an arsenite-activated ATPase (*arsA*) in strain DS-1. Despite its capability to reduce As(V), no open reading frame linked to As(V) reductase (*arsC/arrAB*) was identified within its draft genome (Table S1). Moreover, the draft genome of strain DS-1 harbored the gene cluster essential for dissimilatory-type sulfite reductase, highlighting the presence of crucial genes associated with sulfate reduction (Table S1). Nonetheless, while iron reduction activity was detected in cultures, strain DS-1 sets itself apart from several dissimilatory iron-reducing bacteria like *Geobacter* and *Shewanella* spp. by lacking c-type cytochromes crucial for Fe(III) reduction in those organisms (Weber et al., 2006).

4.2. Microbial anaerobic reduction of ferrihydrite

In order to assess the mechanism of Fe(III) mineral reduction in strain DS-1, the growth rates were measured during its growth on ferrihydrite. Strain DS-1 could effectively utilize lactate as electron donor and ferrihydrite as electron acceptor for Fe(III) reduction, while ferrihydrite reduction was negligible in the lactate-free controls (Fig. 2a). The maximum amounts of Fe(II) and cell protein were obtained on the 30th day (Fig. 2b), during which approximately 40 % of Fe(III) was reduced. The results indicate that strain DS-1 is capable of deriving energy for its growth by coupling the oxidation of lactate with the dissimilatory reduction of ferrihydrite.

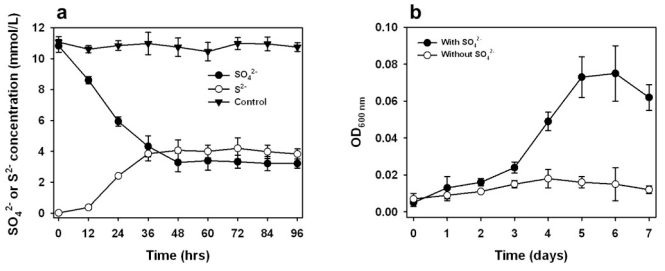


Fig. 1. (a) Respiratory sulfate reduction and (b) growth of *Desulfovibrio* sp. DS-1 on lactate as the electron donor and carbon source. The data are means ± SE ($n = 3$).

4.3. Microbial anaerobic arsenate reduction

When strain DS-1 was cultivated in a lactate-sulfate medium supplemented with 100 μmol/L of As(V), As(III) concentrations increased over 72 h, reaching 15.33 μmol/L in the culture (Fig. 3a). In contrast, As (III) remained undetectable in the abiotic control (Fig. 3b). We further investigated the potential of strain DS-1 to grow and reduce As(V) as the sole electron acceptor. Neither growth nor As(V) reduction was observed under these conditions (data not shown), suggesting that strain DS-1 reduces As(V) via a detoxification pathway rather than respiration. Under anoxic sulfate-reducing conditions, biogenic S^{2-} may form As—S minerals with soluble thioarsenate (As(V)-S) as intermediate, thus removing As from the solution (Fisher et al., 2008). The abiotic reduction of As(V) by biogenic S^{2-} can be excluded, as this reaction is kinetically unfavorable under circumneutral pH conditions (Rochette et al., 2000).

4.4. The effect of microbial Fe(III) reduction and sulfate reduction on arsenic mobility

The impact of strain DS-1-mediated Fe(III) reduction on the geochemical behavior of As in synthetic As(V)-bearing ferrihydrite was examined (Fig. 4). In treatments inoculated with strain DS-1, microbial Fe(III) reduction led to substantial increases in aqueous As concentrations within the first 16 d. At the same time, the concentration of dissolved Fe(II) increased and reached 2.15 mmol/L by 28 d (Fig. 4a). Furthermore, the concentrations of dissolved As(III) and As(V), as well as their increase rates, were higher in the strain DS-1-inoculated treatments compared to the non-inoculated control (Fig. 4b and c). This suggests that the presence of strain DS-1 resulted in increased concentrations of As(III) and As(V) in the cultures. At day 16, As(III) accounted for 79.2 % of the total dissolved As in the strain DS-1-inoculated treatments (Fig. 4c). However, the concentrations of As(III) decreased after this point, indicating that adsorption of As onto ferrihydrite formed through secondary mineralization may have occurred.

To assess the impact of SO_4^{2-} on the fate of As(V) during microbial reduction of Fe(III) in As(V)-bearing ferrihydrite, a series of experiments were conducted in the presence of SO_4^{2-} (Fig. 4). The concentration of As species over time with the addition of SO_4^{2-} is illustrated in Fig. 4b and c. In the anaerobic sulfate-reducing conditions, dissolved Fe(II) concentrations increased to 3.69 mmol/L, 12 d after the first addition of SO_4^{2-} and lactate. As(III) concentrations increased to 19.58 μmol/L by day 12 and decreased to 10.43 μmol/L by day 28. As(V) concentrations in the cultures inoculated with strain DS-1 exhibited minimal variation throughout the experiment, regardless of the addition of SO_4^{2-}. In contrast, the inactivated strain DS-1 cells did not enhance As release, the aqueous As concentrations in the cell-free abiotic control treatments remained relatively constant at 1.07–1.85 μmol/L regardless of the addition of SO_4^{2-} (Fig. 4c).

4.5. Characterization of the biogenic Fe mineralogy precipitates

(i) **XPS.** In this study, we observed As(V) dissolution in the presence of strain DS-1 in the cultures with or without sulfate. We also observed the reductive precipitation of As(III), which was confirmed by analyzing the XPS spectra of the resulting precipitates. Specifically, Fig. 5 depicts the XPS spectra of Fe 2p, S 2p, and As 3d peaks for both the synthetic As(V)-bearing

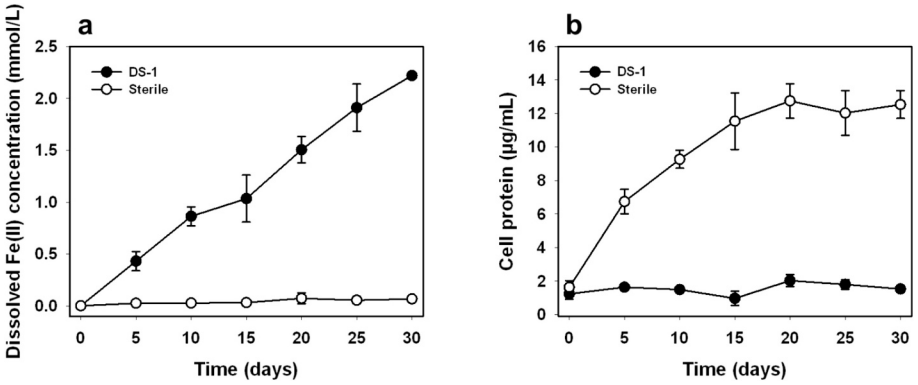


Fig. 2. Time course of anaerobic ferrihydrite reduction (a) and growth (b) by *Desulfovibrio* sp. DS-1 in FWM medium containing 5 mmol/L ferrihydrite. Lactate (10 mmol/L) was used as the electron donor and carbon source. The data are means ± SE (n = 3).

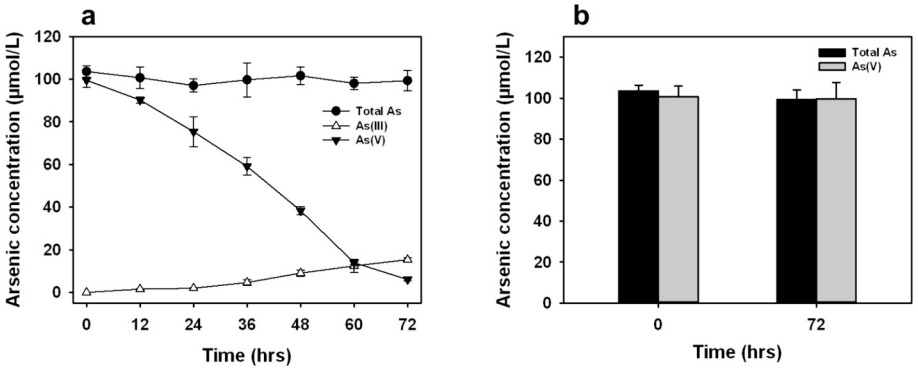


Fig. 3. Time course of anaerobic As(V) reduction in lactate-sulfate medium with an initial As(V) concentration of 100 μmol/L by *Desulfovibrio* sp. DS-1(a), and the arsenic species in the control treatments (b). The data are means ± SE (n = 3).

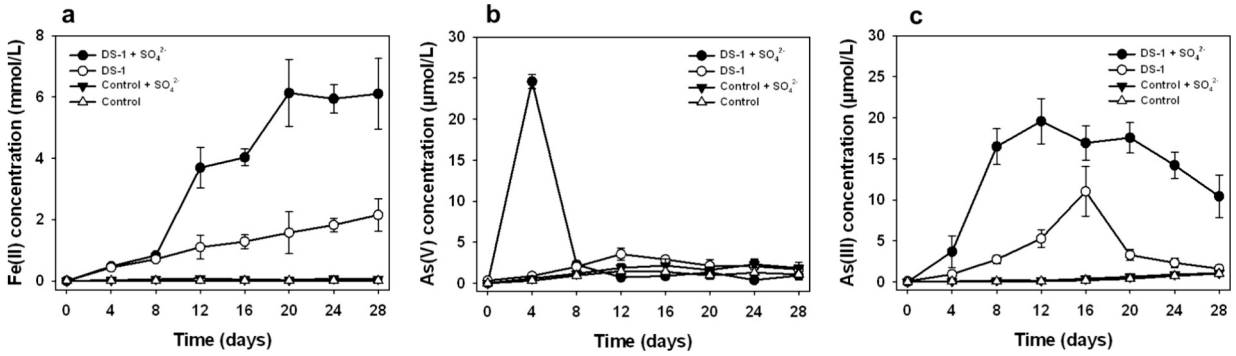


Fig. 4. Concentrations of aqueous Fe(II)(a), As(V)(b), As(III)(c) over time in the As(V)-adsorbed ferrihydrite microbial Fe(III)-reduction experiment with or without sulfate. The aqueous As(III) concentrations were below detection in the abiotic (control) treatments and are therefore not shown. The data are means ± SE (n = 3).

ferrihydrite and the secondary minerals formed in the strain DS-1 treatments. The Fe 2p peak was deconvoluted into Fe(III) and Fe (II) component peaks, while the As 3d peak was deconvoluted into As(V) and As(III) component peaks. By analyzing the area of each component peak, the changes in the oxidation state of Fe and As atoms can be identified. In the Fe 2p spectrum of As(V)-bearing ferrihydrite, four peaks were observed at binding energies of 711.5, 712.2, 713.3, and 714.8 eV. Following deconvolution, curves at binding energies of 707.6, 708.0, 710.1, and 710.6 eV were assigned to Fe(II). These results indicated that all the Fe on the surface of As(V)-bearing ferrihydrite existed in the form of Fe(III).

The injection of strain DS-1 alone (without sulfate) had efficiently promoted the reduction of Fe(III) to Fe(II) within the As(V)-bearing ferrihydrite, with 36.8 % of Fe(III) (724.6 eV) and 63.2 % of Fe(II) (711.0 eV) (Fig. 5a and b). This binding mechanism of Fe(II) and Fe(III) was mainly attributed to the form of Fe-O-C (532.9 eV), Fe-OH (531.8 eV) and Fe—O (530.7 eV) in the As(V)-bearing ferrihydrite that was obtained at the end of the experiment (Fig. 5c). Importantly, anaerobic growth of strain DS-1 showed reductive transformation of As(V) (19.2 % left) to the poorly adsorbed As(III) (80.8 % detected) on the surface of the residual mineral, which was corresponding to the effective increase in As(III) in the microbial solution experiment (Fig. 4c and 5d).

During microbial sulfidization of As(V)-bearing ferrihydrite, SO_4^{2-} was microbially reduced to S^{2-} by SRB. S^{2-} is a strong reductant of Fe

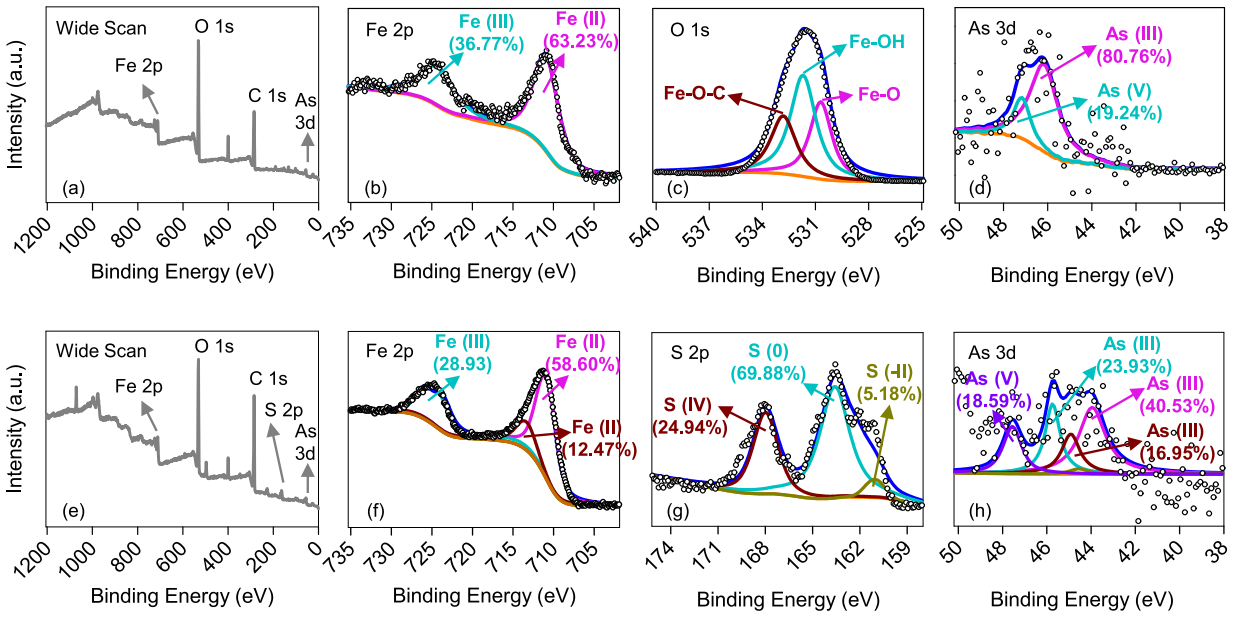


Fig. 5. XPS spectra in O 1 s, S 2p, Fe 2p and As 3d of the As(V)-bearing ferrihydrite and the secondary minerals in *Desulfovibrio* sp. DS-1 inoculation treatments in the absence(a-d) or presence(e-h) of sulfate.

(III) (oxyhydr)oxides and can reduce Fe(III) to Fe(II), thereby forming S^0. The extra addition of SO_4^{2-} had accelerated the reduction of Fe(III) on the mineral surface to Fe(II) by driving the electron transfer from S^{2-} to Fe(III), with the binding energy of 725.3 and 713.6–711.0 eV assigned to Fe(III) (28.9 %) and the two Fe(II) species (71.1 % in total), respectively (Fig. 5e and f). Additionally, we detected three S species [S(IV), S(0) and S(-II)] on the mineral, with the area accounting for 24.9 %, 69.9 % and 5.2 %, respectively (Fig. 5g). This might result in the generation of FeS, which gradually turned the microbial solution color to black (data not shown). In the microbial solution system, either FeS or S^{2-} played a role in the reduction of As(V), resulting in the re-distribution of As including 81.4 % As(III) and 18.6 % As(V) (Fig. 5h). The XPS spectra analysis revealed that during the microbial sulfidization of As(V)-bearing ferrihydrite, some of the As(V) present on the solid surface was partially reduced to As(III).

(ii) XRD spectra.

XRD analysis was employed to investigate the formation of secondary minerals that formed during the reductive dissolution of As(V)-ferrihydrite ($Fh_{As(V)}$) by strain DS-1. In the abiotic treatments, the XRD patterns remain unchanged change after 28 d of incubation. Therefore, no transformation of ferrihydrite to secondary minerals was monitored in the corresponding abiotic control treatments (data not shown). In the biotic strain DS-1 + $Fh_{As(V)}$ treatments, secondary minerals comprising vivianite was detected on the 27th day (Fig. 6). This transformation was attributed to the interaction of Fe^{2+} and PO_4^{3-} in the medium, consistent with prior findings (Islam et al., 2005).

In the strain DS-1 + $Fh_{As(V)}$ + SO_4^{2-} treatments, we did not detect any other secondary minerals, such as vivianite or arsenic sulfides, which are known to form under similar conditions. This absence could potentially be attributed to the low crystallinity of biogenic mackinawite (with the principal component FeS) (Fig. 6).

(iii) TEM/SAED.

To further investigate the potential formation of mackinawite during the microbial reduction, the mineral particles obtained were dispersed onto a formvar−carbon-reinforced nickel grid (300 mesh). Subsequently, the morphology and composition of those particles were

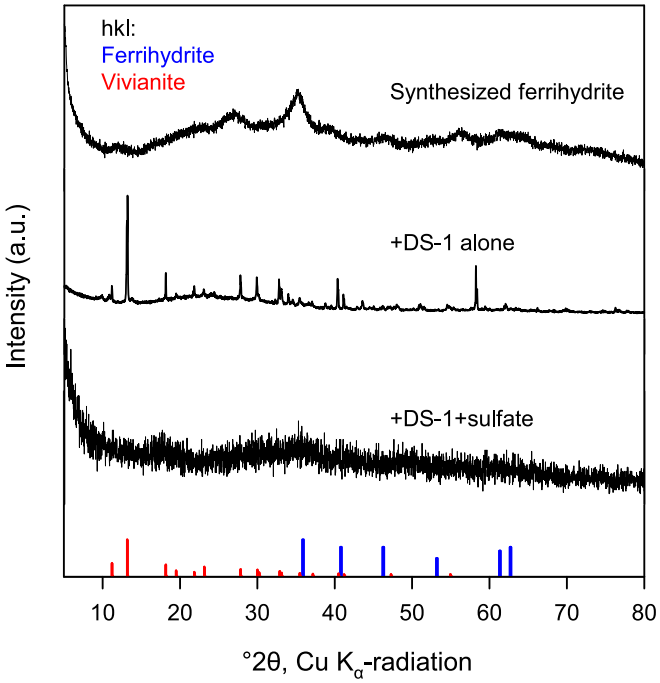


Fig. 6. X-ray diffraction spectra for biogenic Fe minerals produced by *Desulfovibrio* sp. DS-1 in the absence and presence of sulfate. Reference plots for ferrihydrite and vivianite are from JADE 3.1 (Materials Data Inc., Livermore, CA).

analyzed using transmission electron microscope equipped with an energy dispersive spectrometer (TEM-EDS). Further, the mineral species and crystallinity of particles were analyzed using the selected area electron diffraction (SAED). The characterization results revealed the presence of distinct nano-scale granular aggregates at the end of the incubation (Fig. 7a and b). SAED analysis identified a specific group of crystal indices, including (111), (113) and (203), indicative of the crystal structure characteristics of FeS (Fig. 7c). Subsequent EDS analysis corroborated the presence of prominent peaks corresponding to Fe

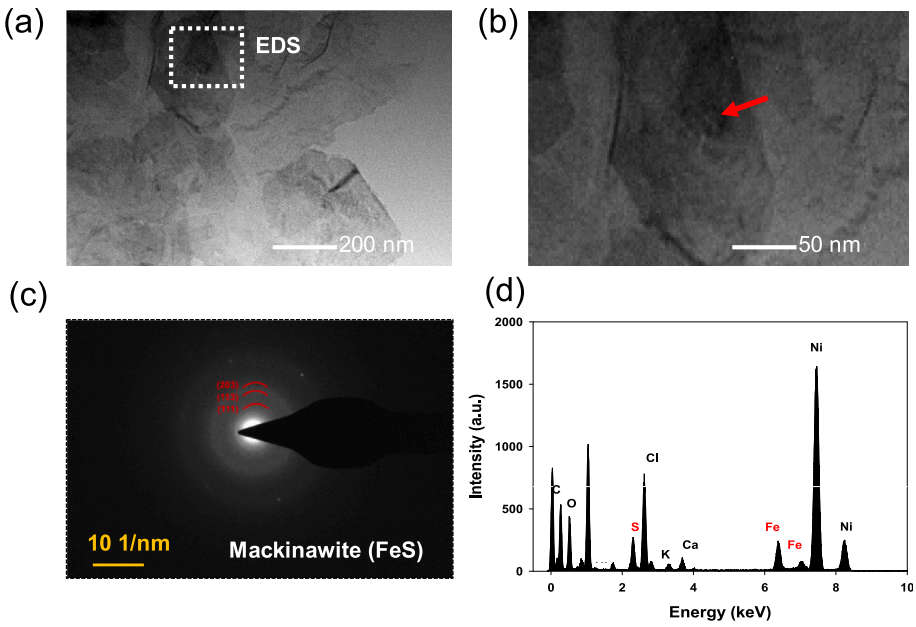


Fig. 7. (a -c) Representative TEM images showing FeS precipitates present in the bulk medium. (d) EDS analysis of areas labeled by arrows in the micrograph above.

and S (Fig. 7d). These results evidenced that strain DS-1 could efficiently facilitate the reduction of Fe(III) (oxyhydr)oxides and SO_4^{2-}, thereby influencing the fate of As(V) by mediating the direct electron transfer to As(V) and/or the indirect electron transfer, with the latter potentially influenced by the intensity of intermediate S^{2-} and FeS.

4.6. *Strain DS-1 promoted arsenic release in paddy soil*

To obtain a comprehension understanding of the metabolic versatility of strain DS-1, we examined its physiological characteristics, focusing on its ability to release As from the soil. We incubated a gamma-irradiated sterile soil slurry with lactate and strain DS-1. In the control without strain DS-1, As(III) was the dominant As species, accounting for 77.2 % and 81.3 % of the total As in the soil porewater and the phosphoric acid extractable fraction, respectively (Fig. 8a and c). However, in the strain DS-1 inoculated treatment, the concentrations of Fe(II), As(III) and total As in the pore water increased by 1.7- fold, 2.7-fold, and 2.3- fold, respectively, compared to the control. Moreover, the concentrations of sulfate in the pore water decreased by 98.2 % in the strain DS-1 inoculated treatment compared to the control, indicating that the sulfate in the porewater was reduced by strain DS-1. The concentrations of phosphoric acid extractable As(V) decreased by 58.8 %, suggesting that strain DS-1 was able to reduce As(V) adsorbed by the soil minerals.

5. Discussion

The accumulation of As in rice is a global food safety concern since rice is a staple food (Meharg and Zhao, 2012). Microbial processes in anoxic paddy environments play a role in the cycling of As and other metal(loid)s (Yu et al., 2016). One important process involves the microbial reduction of Fe(III) (oxyhydr)oxides, leading to the formation of secondary Fe(III)- and Fe(II)-bearing minerals like goethite, lepidocrocite, and magnetite (Hansel et al., 2003; Tufano and Fendorf, 2008). These abundant Fe(III) (oxyhydr)oxides in flooded paddy soils have significant implications for geochemical processes (Xu et al., 2017). Reduction of these Fe(III) (oxyhydr)oxides occurs through biotic and abiotic pathways involving chemical reductants such as sulfide, ascorbic acid, oxalate, and hydroquinones (Afonso and Stumm, 1992; Burton et al., 2011b; Ferris, 2005). Sulfide served as a chemical reductant for Fe(III) (oxyhydr)oxides, facilitating their dissolution, and also acts as a ligand that interact with released Fe(II) to form FeS minerals (Bao et al., 2018). The presence of sulfide alongside Fe(III) (oxyhydr) oxides in soils greatly impacts the biogeochemical transformation of As.

Desulfovibrio species is able to utilize a wide range of electron acceptors, including Fe(III), nitrate, fumarate, and sulfate (Demin et al., 2024). In addition, several *Desulfovibrio* species have been found to be capable of As(V) reduction under anoxic conditions (Li and Krumholz, 2007). In this study, a dissimilatory sulfate- and Fe(III)-reducing bacterium strain DS-1, which belongs to the *Desulfovibrio* genus, was

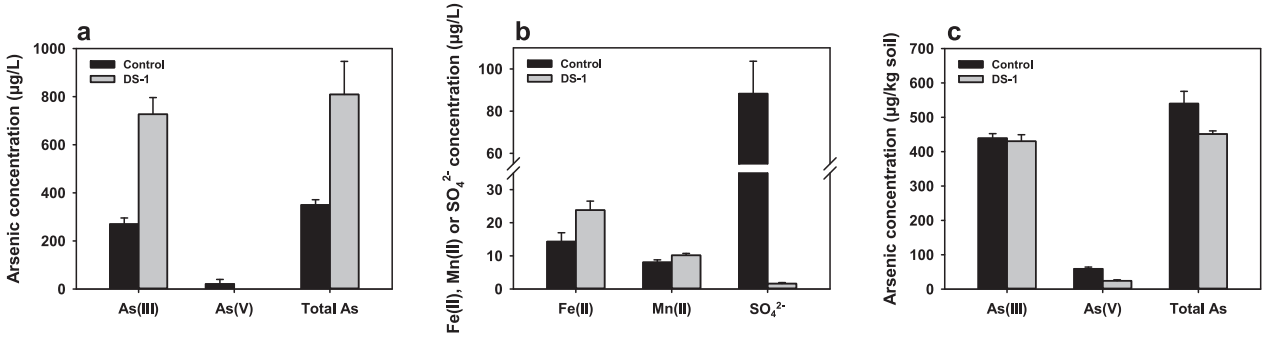


Fig. 8. Effect of *Desulfovibrio* sp. DS-1 inoculation on arsenic speciation (a), Fe(II), Mn(II), and SO_4^{2-} (b) in the soil solution and the phosphoric acid-extractable arsenic fraction (c) in a flooded soil slurry. Control, control without DS-1 inoculation; DS-1, with DS-1 inoculum. The data are means ± SE (n = 3).

isolated. Strain DS-1 was capable of reducing As(V) to As(III) under anoxic sulfate-reducing conditions. Importantly, it was observed that strain DS-1 could not grow using As(V) as the sole electron acceptor, indicating that it reduces As(V) as a detoxification mechanism rather than for respiration. In the absence of sulfate, strain DS-1 was able to directly reduce ferrihydrite, leading to the formation of vivianite and a decrease in aqueous As concentration. However, anaerobic sulfate reduction had multiple effects on the fate of As. Our study revealed that the increased in SO_4^{2-} concentration facilitated the transformation from ferrihydrite to mackinawite. This transformation occurred as the produced S^{2-} could co-precipitate with dissolved Fe^{2+}, leading to the formation of mackinawite (Gao et al., 2021). Moreover, the addition of sulfate lead to the release of a portion of As(V) into the solution, which was subsequently reduced to As(III). During these processes, desorption and re-precipitation of As occur simultaneously. Due to its strong affinity for poorly crystalline Fe(III) (oxyhydr)oxides, arsenic preferentially re-adsorbed onto residual ferrihydrite.

Previous studies have been conducted to explore the relationship between bacterial sulfate reduction and the mobilization of As, primarily through laboratory studies (Kocar et al., 2010; Saalfield and Bostick, 2009; Zhu et al., 2008). However, the natural redox system in As-containing paddy soils is complex and dynamic, involving the cycling of Fe, S, and As. When strain DS-1 cells were inoculated to the soil slurry, the concentrations of Fe(II), As(III), and total As in the pore water increased compared to the control treatment. This suggests that strain DS-1 was able to mobilize As in the flooded paddy environment. Additionally, strain DS-1 was found to be capable of reducing As(V) to As(III) in the soil, as indicated by the decrease in phosphoric acid extractable As (V) concentrations. This suggests that strain DS-1 can reduce As(V) to As (III) in both liquid and solid phases. Despite the absence of the As(V) reductase *arsC* or *arr* gene in strain DS-1, it is conceivable that it may possess a novel As(V) reductase gene involved in the reduction of As(V).

In this study, the isolation of strain DS-1 holds significance as it provides valuable insights into the mechanisms behind As mobilization in paddy soils under anoxic sulfate-reducing conditions. The ability of strain DS-1 to mediate the reductive dissolution of Fe(III) (oxyhydr) oxides is believed to play a role in the mobilization of As in paddy soils. This research conducted on strain DS-1 has not only highlighted the importance of microbial sulfate and Fe(III) reduction but also shed light on its crucial role in mobilizing As from soil.

6. Conclusions

The paddy environment is a new frontier in understanding the phylogenetic diversity and influence of As-metabolizing microorganisms, even though their abundance is well-documented in other places. This study presents the first instance of an anaerobic sulfate-reducing bacterium isolated from a paddy soil that reduces Fe(III) and sulfate and shows the potential for microbiological As redox cycling within such an environment. The discovery of strain DS-1 significantly fills a current knowledge gap on sulfate-reducing prokaryotes in paddy environments. Given that As is often found in association with iron minerals in paddy soils, the ability of strain DS-1-like bacteria to mobilize As could have a significant impact on the biogeochemical transformation of As.

CRediT authorship contribution statement

Yi-Fei Wu: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jun Zhang:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition. **Gang Hu:** Writing – review & editing, Validation. **Jiajia Wang:** Writing – review & editing, Project administration. **Chao Peng:** Writing – review & editing, Supervision. **Andreas Kappler:** Writing – review & editing, Validation. **Fang-Jie Zhao:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This project was supported by National Natural Science Foundation of China (Grant No. 32370129 to J.Z, No. 42107010 to J.W).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176529.

References

Aeppli, M., Vranic, S., Kaegi, R., Kretzschmar, R., Brown, A.R., Voegelin, A., et al., 2019. Decreases in iron oxide reducibility during microbial reductive dissolution and transformation of ferrihydrite. Environ. Sci. Technol. 53, 8736–8746.
Afonso, M.D., Stumm, W., 1992. Reductive dissolution of iron(III) (hydr)oxides by hydrogen-sulfide. Langmuir 8, 1671–1675.
Andres, J., Bertin, P.N., 2016. The microbial genomics of arsenic. FEMS Microbiol. Rev. 40, 299–322.
Bao, P., Li, G.X., Sun, G.X., Xu, Y.Y., Meharg, A.A., Zhu, Y.G., 2018. The role of sulfate-reducing prokaryotes in the coupling of element biogeochemical cycling. Sci. Total Environ. 613, 398–408.
Burton, E.D., Bush, R.T., Johnston, S.G., Sullivan, L.A., Keene, A.F., 2011a. Sulfur biogeochemical cycling and novel Fe-S mineralization pathways in a tidally re-flooded wetland. Geochim. Cosmochim. Acta 75, 3434–3451.
Burton, E.D., Johnston, S.G., Bush, R.T., 2011b. Microbial sulfidogenesis in ferrihydrite-rich environments: effects on iron mineralogy and arsenic mobility. Geochim. Cosmochim. Acta 75, 3072–3087.
Chen, C., Li, L.Y., Huang, K., Zhang, J., Xie, W.Y., Lu, Y.H., et al., 2019. Sulfate-reducing bacteria and methanogens are involved in arsenic methylation and demethylation in paddy soils. ISME J. 13, 2523–2535.
Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14, 454.
Dai, J., Tang, Z., Jiang, N., Kopittke, P.M., Zhao, F.-J., Wang, P., 2020. Increased arsenic mobilization in the rice rhizosphere is mediated by iron-reducing bacteria. Environ. Pollut. 263, 114561.
Demin, K.A., Prazdnova, E.V., Minkina, T.M., Gorovtsov, A.V., 2024. Sulfate-reducing bacteria unearthed: ecological functions of the diverse prokaryotic group in terrestrial environments. Appl. Environ. Microbiol. 90 (4), e01390-23.
Dixit, S., Hering, J.G., 2003. Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: implications for arsenic mobility. Environ. Sci. Technol. 37, 4182–4189.
Fang, X., Christl, I., Blanco, A.E.C., Planer-Friedrich, B., Zhao, F.-J., Kretzschmar, R., 2023. Decreasing arsenic in rice: interactions of soil sulfate amendment and water management. Environ. Pollut. 322, 121152.
Fang, X., Blanco, A.E.C., Christl, I., Le Bars, M., Straub, D., Kleindienst, S., et al., 2024. Simultaneously decreasing arsenic and cadmium in rice by soil sulfate and limestone amendment under intermittent flooding. Environ. Pollut. 123786.
Ferris, F.G., 2005. Biogeochemical properties of bacteriogenic iron oxides. Geomicrobiol J. 22, 79–85.
Fisher, J.C., Wallschlager, D., Planer-Friedrich, B., Hollibaugh, J.T., 2008. A new role for sulfur in arsenic cycling. Environ. Sci. Technol. 42, 81–85.
Gao, K., Hu, Y., Guo, C.L., Ke, C.D., Lu, G.N., Dang, Z., 2021. Mobilization of arsenic during reductive dissolution of as(V)-bearing jarosite by a sulfate reducing bacterium. J. Hazard. Mater. 402.
Giral, M., Zagury, G.J., Deschênes, L., Blouin, J.-P., 2010. Comparison of four extraction procedures to assess arsenate and arsenite species in contaminated soils. Environ. Pollut. 158, 1890–1898.
Hansel, C.M., Benner, S.G., Neiss, J., Dohnalkova, A., Kukkadapu, R.K., Fendorf, S., 2003. Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. Geochim. Cosmochim. Acta 67, 2977–2992.
He, L.Y., Xie, L., Wang, D.J., Li, W.L., Fortner, J.D., Li, Q.Q., et al., 2019. Elucidating the role of sulfide on the stability of ferrihydrite colloids under anoxic conditions. Environ. Sci. Technol. 53, 4173–4184.
Islam, F.S., Gault, A.G., Boothman, C., Polya, D.A., Charnock, J.M., Chatterjee, D., Lloyd, J.R., 2004. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature 430, 68–71.
Islam, F.S., Pederick, R.L., Gault, A.G., Adams, L.K., Polya, D.A., Charnock, J.M., Lloyd, J. R., 2005. Interactions between the Fe(III)-reducing bacterium *Geobacter*

sulfurreducens and arsenate, and capture of the metalloid by biogenic Fe(II). Appl. Environ. Microbiol. 71, 8642–8648.
Kirk, G., 2004. The Biogeochemistry of Submerged Soils. John Wiley & Sons, Ltd.
Kocar, B.D., Borch, T., Fendorf, S., 2010. Arsenic repartitioning during biogenic sulfidization and transformation of ferrihydrite. Geochim. Cosmochim. Acta 74, 980–994.
Kubeneck, L.J., Notini, L., Rothwell, K.A., Fantappiè, G., Huthwelker, T., ThomasArrigo, L.K., Kretzschmar, R., 2024. Transformation of vivianite in intertidal sediments with contrasting sulfide conditions. Geochim. Cosmochim. Acta 370, 173–187.
Kumar, N., Noël, V., Planer-Friedrich, B., Besold, J., Lezama-Pacheco, J., Bargar, J.R., et al., 2020. Redox heterogeneities promote thioarsenate formation and release into groundwater from low arsenic sediments. Environ. Sci. Technol. 54, 3237–3244.
Langner, H.W., Inskeep, W.P., 2000. Microbial reduction of arsenate in the presence of ferrihydrite. Environ. Sci. Technol. 34, 3131–3136.
Li, G., Sun, G.X., Williams, P.N., Nunes, L., Zhu, Y.G., 2011. Inorganic arsenic in Chinese food and its cancer risk. Environ. Int. 37, 1219–1225.
Li, X.K., Krumholz, L.R., 2007. Regulation of arsenate resistance in *Desulfovibrio desulfuricans* G20 by an *arsRBCC* operon and an *arsC* gene. J. Bacteriol. 189, 3705–3711.
Lovley, D.R., 1993a. Anaerobes into heavy-metal - dissimilatory metal reduction in anoxic environments. Trends Ecol. Evol. 8, 213–217.
Lovley, D.R., 1993b. Dissimilatory metal reduction. Ann. Rev. Microbiol. 47, 263–290.
Ma, X., Wang, S., Gomez, M.A., Yuan, Z., Wu, X., Yao, S., Jia, Y., 2022. Insight into the effect of $SO4^{2-}$ on the precipitation and solubility of ferric arsenate in acidic solutions: implication for arsenic mobility and fate. Chem. Geol. 602, 120900.
Macy, J.M., Santini, J.M., Pauling, B.V., O'Neill, A.H., Sly, L.I., 2000. Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Arch. Microbiol. 173, 49–57.
Meharg, A.A., Zhao, F.J., 2012. Arsenic & Rice. Springer, Dordrecht, p. p171.
Muehe, E.M., Morin, G., Scheer, L., Le Pape, P., Esteve, I., Daus, B., Kappler, A., 2016. Arsenic(V) incorporation in vivianite during microbial reduction of arsenic(V)-bearing biogenic Fe(III) (oxyhydr)oxides. Environ. Sci. Technol. 50, 2281–2291.
Oremland, R.S., Stolz, J.F., 2003. The ecology of arsenic. Science 300, 939–944.
Rochette, E.A., Bostick, B.C., Li, G., Fendorf, S., 2000. Kinetics of arsenate reduction by dissolved sulfide. Environ. Sci. Technol. 34, 4714–4720.
Saalfield, S.L., Bostick, B.C., 2009. Changes in iron, sulfur, and arsenic speciation associated with bacterial sulfate reduction in ferrihydrite-rich systems. Environ. Sci. Technol. 43, 8787–8793.
Saltikov, C.W., Newman, D.K., 2003. Genetic identification of a respiratory arsenate reductase. Proc. Natl. Acad. Sci. USA 100, 10983–10988.
Schwertmann, U., Cornell, R.M., 2000. Iron Oxides in the Laboratory: Preparation and Characterization, 2nd ed. Wiley-VCH Verlag GmbH, Weinheim, Germany.
Shen, J., Song, X.W., Bickel, D., Rosen, B.P., Zhao, F.J., Messens, J., Zhang, J., 2024. Arsenate reductase of *Rufibacter tibetensis* is a metallophosphoesterase evolved to catalyze redox reactions. Mol. Microbiol. 122, 201–212.
Silver, S., Phung, L.T., 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl. Environ. Microbiol. 71, 599–608.
Stookey, L.L., 1970. Ferrozine-a new spectrophotometric reagent for iron. Anal. Chem. 42, 779–781.
Takahashi, Y., Minamikawa, R., Hattori, K.H., Kurishima, K., Kihou, N., Yuita, K., 2004. Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. Environ. Sci. Technol. 38, 1038–1044.
ThomasArrigo, L.K., Bouchet, S., Kaegi, R., Kretzschmar, R., 2020. Organic matter influences transformation products of ferrihydrite exposed to sulfide. Environ. Sci. Nano 7, 3405–3418.
Tseng, W.P., Chu, H.M., How, S.W., Fong, J.M., Lin, C.S., Yeh, S., 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J. Natl. Cancer Inst. 40, 453–463.
Tufano, K.J., Fendorf, S., 2008. Confounding impacts of iron reduction on arsenic retention. Environ. Sci. Technol. 42, 4777–4783.
Wang, M., Tang, Z., Chen, X.P., Wang, X., Zhou, W.X., Tang, Z., et al., 2019. Water management impacts the soil microbial communities and total arsenic and methylated arsenicals in rice grains. Environ. Pollut. 247, 736–744.
Wang, Q., Wang, J., Wang, X., Kumar, N., Pan, Z., Peiffer, S., Wang, Z., 2023. Transformations of ferrihydrite–extracellular polymeric substance coprecipitates driven by dissolved sulfide: interrelated effects of carbon and sulfur loadings. Environ. Sci. Technol. 57, 4342–4353.
Weber, K.A., Achenbach, L.A., Coates, J.D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. Nat. Rev. Microbiol. 4, 752–764.
Xu, X., Chen, C., Wang, P., Kretzschmar, R., Zhao, F.-J., 2017. Control of arsenic mobilization in paddy soils by manganese and iron oxides. Environ. Pollut. 231, 37–47.
Xu, X.W., Wang, P., Zhang, J., Chen, C., Wang, Z.P., Kopittke, P.M., et al., 2019. Microbial sulfate reduction decreases arsenic mobilization in flooded paddy soils with high potential for microbial Fe reduction. Environ. Pollut. 251, 952–960.
Yin, Z., Ye, L., Jing, C., 2022. Genome-resolved metagenomics and metatranscriptomics reveal that Aquificae dominates arsenate reduction in Tengchong geothermal springs. Environ. Sci. Technol. 56, 16473–16482.
Yu, H.-Y., Li, F.-B., Liu, C.-S., Huang, W., Liu, T.-X., Yu, W.-M., 2016. Iron redox cycling coupled to transformation and immobilization of heavy metals: implications for paddy rice safety in the red soil of South China. Adv. Agron. 137, 279–317.
Zhang, J., Zhou, W., Liu, B., He, J., Shen, Q., Zhao, F.J., 2015. Anaerobic arsenite oxidation by an autotrophic arsenite-oxidizing bacterium from an arsenic-contaminated paddy soil. Environ. Sci. Technol. 49, 5956–5964.
Zhang, J., Zhao, S., Xu, Y., Zhou, W., Huang, K., Tang, Z., Zhao, F.J., 2017. Nitrate stimulates anaerobic microbial arsenite oxidation in paddy soils. Environ. Sci. Technol. 51, 4377–4386.
Zhao, Z.X., Wang, S.F., Jia, Y.F., 2017. Effect of sulfide on as(III) and as(V) sequestration by ferrihydrite. Chemosphere 185, 321–328.
Zhu, W., Young, L.Y., Yee, N., Serfes, M., Rhine, E.D., Reinfelder, J.R., 2008. Sulfide-driven arsenic mobilization from arsenopyrite and black shale pyrite. Geochim. Cosmochim. Acta 72, 5243–5250.