Arsenate reduction and mobilization in the presence of indigenous aerobic bacteria obtained from high arsenic aquifers of the Hetao basin, Inner Mongolia

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Abstract

Intact aquifer sediments were collected to obtain As-resistant bacteria from the Hetao basin. Two strains of aerobic As-resistant bacteria (Pseudomonas sp. M17-1 and Bacillus sp. M17-15) were isolated from the aquifer sediments. Those strains exhibited high resistances to both As(III) and As(V). Results showed that both strains had arr and ars genes, and led to reduction of dissolved As(V), goethite-adsorbed As(V), scorodite As(V) and sediment As(V), in the presence of organic carbon as the carbon source. After reduction of solid As(V), As release was observed from the solids to solutions. Strain M17-15 had a higher ability than strain M17-1 in reducing As(V) and promoting the release of As. These results suggested that the strains would mediate As(V) reduction to As(III), and thereafter release As(III), due to the higher mobility of As(III) in most aquifer systems. The processes would play an important role in genesis of high As groundwater.

1. Introduction

Arsenic is one of the most common and harmful carcinogens in the environment. The problem of As contamination in groundwater has become a worldwide environmental issue (Ravenscroft et al., 2009). It directly endangers human health, and influences the sustainable development of society. Therefore, the source and fate of groundwater As is one of the hottest topics in the field of environmental science. Several biogeochemical processes control the release and transport of As in natural waters, including adsorption, oxidation-reduction, and microbe-mediated electron transfer (Diesel et al., 2009). More and more studies have shown that microbes play an important role in the release, migration and transformation of As in aqueous systems (Oremland and Stolz, 2005; Anderson and Cook, 2004; Chang et al., 2008; Pepi et al., 2007; Lievremont et al., 2009; Chang et al., 2012; Mirza et al., 2014).

Although As is generally toxic to life, a lot of microorganisms can get the energy for growth through metabolizing As (Oremland and Stolz, 2003). These microorganisms have evolved the necessary genetic components which confer resistance mechanisms, including arsenite-oxidation, arsenate-reduction and As(V) resistance minimizing the amount of As that enters the cells (Cervantes et al., 1994; Ji and Silver, 1992a,b). Additionally, the microorganisms can use As compounds as electron donors or electron acceptors, and possess As detoxification mechanisms (Ahmann et al., 1994; Johnson et al., 2003). These microorganisms are taxonomically diverse and metabolically versatile, mainly including the dissipatory arsenate-respiring prokaryotes (DARPs), chemoaotrophic arsenite-oxidizing bacteria (CAOs), heterotrophic arsenite-oxidizing bacteria (HAOs), and arsenate-resistant microbes (Oremland and Stolz, 2005; Anderson and Cook, 2004; Chang et al., 2008; Pepi et al., 2007). Since As(III)-oxidizing bacteria have firstly been found by Green (1918), a series of As(III)-oxidizing bacteria have been isolated (Anderson et al., 1992; Santini et al., 2000; Kashyap et al., 2006; Muller et al., 2007; Duquesne et al., 2008).

Arsenic(V)-reducing bacteria are one of the dominant bacterial groups involved in the cycle of As (Lievremont et al., 2009). A series of As(V)-reducing bacteria have been isolated in previous studies (Santini et al., 2004; Handley et al., 2009; Chang et al., 2012;
Anderson and Cook, 2004; Liao et al., 2011). Microbial As(V) reduction occurs via two common mechanisms, respiration and detoxification. Respiration occurs only under anaerobic conditions. During the respiration, the arrA gene, which encodes for a reductase, catalyzes respiratory As(V) reduction (Saltikov and Newman, 2003; Affar et al., 2003; Murphy and Saltikov, 2009). Detoxification is an efficient As(V)-reducing mechanism that occurs under both aerobic and anaerobic conditions (Silver and Phung, 2005; Murphy and Saltikov, 2009). During the detoxification process, the arsC gene, which is responsible for the biotransformation of As(V) to As(III) (Krumova et al., 2008; Musingarimi et al., 2010), and the arsB, which extrudes As(III) from the cytoplasm (Rosen, 2002; Silver and Phung, 1996), would be involved. Furthermore, organoarsenicals are presumed to be formed via microbial activities (Cullen and Reimer, 1989). Bacteria have a function of As methyl-ation, i.e., having arsenite methylation transfersases that transfer As(III) to As methyl products, such as MMA, DMA (Oremland and Phung, 2005). Previous studies have investigated characteristics of sedi-ment, high As groundwater distribution, chemical and isotopic characteristics, and migration and transformation of groundwater As (Guo et al., 2008b, 2010, 2011a, 2011b, 2012, 2013a, 2013b). However, few studies have been conducted to investigate in-fluences of microbiological processes on As speciation and mobility in the aquifers of the Hetao basin (Li et al., 2014). Li et al. (2014) observed that Pseudomonas, Dietzia and Rhodococcus widely occurred in aquifer systems and anaerobic NO3-reducing bacteria Pseudomonas sp. was the largest group, followed by Fe(III)-reducing, SO42−-reducing and As(V)-reducing bacteria, although bacterial diversity was dependent on groundwater As concentra-tion. However, the roles of indigenous aerobic bacteria in As cycling are unclear and need more investigation. Therefore, it is important to understand As resistance of indigenous aerobic bacteria and their roles in As transformation and mobility. The objectives of this study are to 1) characterize effects of As on indigenous aerobic bacteria from aquifer sediments hosting high As groundwater, 2) evaluate As(V) reduction in As(V)-containing media in the presence of the indigenous aerobic bacteria, and 3) assess As mobilization during bacteria-solid-water interactions.

2. Materials and methods

2.1. Sample collection

Sediment samples M14 and M17 were collected at depths of 12.2 and 25.0 m, respectively, from the borehole (40°58’02″N, 107°00’52″E) in the Hetao basin, Inner Mongolia, in 2009. Imme-diately after removal from the borehole, the sediment samples were put into sterilized plastic bags and sealed under pure N2 gas (N2 > 99.999%). They were transported at 4°C to the laboratory, and stored in a refrigerator at −20°C for experiments. M17 is gray sand, and used for isolation of aerobic As-resistant bacteria, since gray sand universally occurs in aquifers hosting high As groundwater (Guo et al., 2008a). M14 is brown clay with 100% As as As(V) spe-cies, which allowed us to investigate the reduction of sediment As(V). Therefore, it was used as the solid phase in microcosm experiments.

In comparison with M14, more SiO2, but lower Al2O3, Fe2O3, As, F and Mn were observed in sediment M17 (Table 1). Arsenic content was 88.2 and 7.9 mg/kg in M14 and M17, respectively. At the depth around 25 m, groundwater had high As concentrations, with As(III) of 528 μg/L and As(V) of 54.0 μg/L.

2.2. Isolation of aerobic As-resistant bacteria

Aerobic As-resistant bacteria were cultivated in CDM medium (Text 1 in Supplementary Materials). Sediment sample M17 (10 g) was mixed with 3 mL solution with 1.5 mg/L As(III) or As(V), which was filtered by 0.22 µm filter membrane to remove microorganisms beforehand, in 100 mL glass flasks sealed with sterilized air-permeable polypropylene membranes (Thermo Scientific ABgene). The mixtures were incubated for 10 d in a shaking water bath at 150 rpm at 28°C. In order to keep the humidity of samples, sterilized water was added each 1~2 days (Zhao, 2009). After that, 50 mL sterilized water was added to the mixtures. The suspension was incubated for 1 h at 150 rpm at 28°C. The supernatant was diluted to 102~105. For each dilution, 100 μL supernatant was added to CDM plates. The plates were incubated for 2 d at 28°C. Then, single strains were selected and plate streaking method was used to obtain pure bacteria (Liao et al., 2011). The pure strains were stored in 4°C.

2.3. Identification of aerobic As-resistant bacteria

The aerobic As-resistant strains were identified, using the 16S rRNA and As marker gene sequence analysis method. Sequences of 16S rRNA gene and As marker genes are shown in Table 2. After the DNA extraction, bacterial 16S rRNA gene was amplified using bacterial universal primers. Degenerate primers used to amplify the As marker genes were designed especially for arrA, arrB, and arrC. The amplification procedure was provided in details in Text 2 of Supplementary Materials.

The clone sequencing was detected by the Beijing ZhongKe Xilin Biotechnology Company Limited. The DNA sequencing results were analyzed for similarities and aligned in the BLAST program pack-ages (http://blast.ncbi.nlm.nih.gov). The NCBI (USA) was used as the reference database to identify 16S rRNA gene and amplified As marker gene sequences.

2.4. Experimental procedure

The pure strains were cultured in the CDM medium. The strain suspensions were used in microcosm experiments. To examine effects of bacteria on As(V) reduction and mobilization, four As(V) sources were prepared. One is dissolved As(V) with As concentrations of 0.5 and 7.5 mg/L (Treatment I); one is As(V)-adsorbing goethite (α-FeOOH) with As content of 5.6 mg/g (Text 3 in Supplementary Materials) (Treatment II); one is crystalline scorodite (FeAsO4·2H2O) (Treatment III), and the other sediment M14 with As content of 88.2 mg/kg (Treatment IV). In each treatment, blank
batches (free of the strains) were also carried out to investigate effect of the strains, which were sterilized at 121 °C for 20 min. The solid–liquid ratio in Treatments II, III was 0.1 g: 50 mL, and Treatment IV 1 g: 50 mL. The ratio of bacterial suspensions to medium was 1: 50 (w/w), which ensured the similar initial microbial population. All batches were incubated at 28 °C. 

Dissolved As solutions were prepared from sodium arsenite for As(III) and sodium arsenate for As(V). Arsenic(V)-adsorbing goethite was synthesized as described in Text 3 of Supplementary Materials. Crystalline scorodite was obtained from a mineral company in Guizhou province, PR China, which contained 100% scorodite (ODS) was less than 2%. The pH values were measured using pH meter (Sartorius, PB-21), dissolved Fe(II) using spectrophotometer (DR 2800, HACH) with detection limit was 0.1 μg/L, and the relative standard deviation (RSD) was less than ±2%. The detection limit for As(III) and As(V) was 0.2 μg/L, and the relative standard deviation (RDS) was less than ±2%. The pH values were measured using pH meter (Sartorius, PB-21), dissolved Fe(II) using spectrophotometer (DR 2800, HACH) with detection limit was 0.1 μg/L, and the relative standard deviation (RSD) was less than ±2%. The detection limit for As(III) and As(V) was 0.2 μg/L, and the relative standard deviation (RDS) was less than ±2%. The pH values were measured using pH meter (Sartorius, PB-21), dissolved Fe(II) using spectrophotometer (DR 2800, HACH) with detection limit was 0.1 μg/L, and the relative standard deviation (RSD) was less than ±2%. The detection limit for As(III) and As(V) was 0.2 μg/L, and the relative standard deviation (RDS) was less than ±2%

The primer sequences used for the PCR of arrA gene and ars genes.

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<td></td>
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2.5. Analytical techniques

Dissolved As and Fe were analyzed by ICP-MS (7500C, Agilent). Arsenic species (including inorganic As(III), inorganic As(V), MMA and DMA) were determined using an HPLC-ICP-MS. For total As, the detection limit was 0.1 μg/L, and the relative standard deviation (RSD) was less than ±2%. The pH values were measured using pH meter (Sartorius, PB-21), dissolved Fe(II) using spectrophotometer (DR 2800, HACH) with 1, 10 phenanthroline method. Analytical details of sediment samples were provided in Text 4 of Supplementary Materials.

Bacterial growth was monitored by measuring the optical density (OD) of the cultures at 600 nm with spectrophotometer (Shanghai Precision Scientific UV1752N). Concentrations of ATP were determined by ATP fluorescence detector (AF-100, DKK-TOA).

Arsenic K-edge X-ray absorption near-edge structure (XANES) spectra were recorded at room temperature at beamline BL15U1 of the Shanghai Synchrotron Radiation Facility (SSRF), China. Details were provided in Text 4 of Supplementary Materials. Since no dissolved Fe(II) was detected in all incubation batches, Fe K-edge XANES spectra were not recorded for solid samples.

3. Results and discussion

3.1. Characteristics of aerobic As-resistant bacteria

Two strains of aerobic As-resistant bacteria, M17−15 and M17−1,

<table>
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<th>Genes</th>
<th>Isolate</th>
<th>Primer</th>
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<tr>
<td>arrA</td>
<td>+</td>
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<td>arsB</td>
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<td>arsC</td>
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|        |         |        |                                 |           | were isolated and identified. The 16S rRNA gene sequences of the two strains showed the highest similarity to Bacillus flexus strain JMC-UBL 24 (HM451429.1) with 99% similarity and Pseudomonas stutzeri strain Cpa_a4 (Sas, 2009) with 99% similarity, respectively. The high similarity suggested that the bacterial strains M17−15 and M17−1 were Bacillus sp. and Pseudomonas sp., respectively.

The arsC gene was successfully amplified (Table 3). The length of putative arsC gene fragments was 326 bp. Phylogenetic cluster analysis showed that the arsC of strain M17−15 was phylogenetically located close to the arsC of strain M17−1, and their arsC gene sequence was placed together in the cluster of arsC family. The closest arsC sequence of strains M17−15 and M17−1 was uncultured bacterium clone ZJ-arsC-40 arsenate reductase (arsC) gene, with the similarity of 93%. The arsB gene was not observed in these two strains.

The arrA gene was detected in these two isolates (Table 3). A 148 bp putative arrA gene fragment of the two strains were successfully amplified. The sequences of strains M17−15 and M17−1 had the high similarity to uncultured bacterium arrA gene for As(V) respiratory reductase.

3.2. Effect of As on bacterial growth

Fig. 1 shows the growth of strains M17−15 and M17−1 in treatments with different As concentrations. The logarithmic growth of strain M17−15 began at 24 h for all treatments (Fig. 1a). The maximum biomass was reached at around 48 h in controls without As amendment, and at around 72 h in treatments with 0.5 mg/L As(V)/As(III). However, high As treatments with 7.5 mg/L As(V)/As(III)) obtained the maximum biomass at longer incubation time (around 120 h). Although the incubation times obtaining the maximum biomass were different, the maximum biomasses were identical for all treatments. Therefore, addition of As(III) and As(V) had no obvious inhibitory effect on the growth of strain M17−15, although the growth rate was retarded by the presence of As.

Strain M17−1 experienced the logarithmic growth at the incubation time between 12 and 60 h (Fig. 1b). The biomass reached the maximum at around 60 h, although the maximum biomass was different for different treatments. Controls and treatments with 7.5 mg/L As(III) had the largest biomass, followed by treatments with 7.5 mg/L As(V) and treatments with 0.5 mg/L As(V)/As(III). Although As(III) shows 60 times more toxicity than As(V) (Korte and Fernando, 1991), As(III) did not exhibit evident inhibitory effect on the growth of strains in comparison with As(V). Those results indicated that both strain M17−15 and strain M17−1 had a resistance to dissolved As(III) and As(V).

Other study showed that the P. stutzeri strain As-1 isolated in the laboratory grew in a culture medium with 50 mM As(V) or 0.2 mM As(III) (Joshi et al, 2008). Joshi et al. (2008) found that, compared with the growth in the medium with the absence of As, the strain growth in the media with 50 mM As(V) or 0.2 mM As(III) decreased by 43% and 56%. Liao et al. (2011) found that, under aerobic conditions on MSM agar plates, the lowest As(III) concentration that
completely inhibited the growth of the Bacillus sp. (AR-9) and Pseudomonas sp. (AR-2) is 5 mM and 2 mM, respectively, and the lowest As(V) concentration is 200 mM and 100 mM, respectively. Since As concentrations in our experiments were far less than the lowest inhibition concentration, either As(V) or As(III) showed no significantly inhibitory effect on the growth of strains.

3.3. Biotransformation of dissolved As

Results showed that As(III) concentration did not change with time in the presence of strains M17-15 and M17-1 in culture media with As(III) (data not shown). No As(V) was detected, indicating that strains M17-15 and M17-1 did not oxidize dissolved As(III).

However, As(V) was reduced to As(III) in the presence of strain M17-15 or M17-1 in growth media. Fig. 2 shows variations in As species during experiments with strain M17-15 or M17-1 and 0.5 mg/L or 7.5 mg/L As(V). During the experiments, no methyl As products (such as MMA, DMA) were detected. Strain M17-15 reduced As(V) quickly at the beginning of logarithmic growth. The strain M17-15 completely transformed 0.5 mg/L As(V) to As(III) within 6 h (the incubation time between 24 and 30 h) (Fig. 2a).

Initial As(V) concentrations did not have a significant influence on
reduction duration \( p < 0.05 \). When initial As(V) concentration increased to 7.5 mg/L, the time for transformation of all As(V) to As(III) was still 6 h (between 24 and 30 h) (Fig. 2b).

Arsenic(V) was reduced in the batches with the presence of the M17-1 more slowly than in the batches with the presence of the M17-15. All dissolved As(V) was reduced to As(III) by strain M17-1 within 12 h (the incubation time between 12 and 24 h) at initial concentration of 0.5 mg/L (Fig. 2c), and within 24 h at initial concentration of 7.5 mg/L As(V) (the incubation time between 12 and 36 h) (Fig. 2d). Concentrations of ATP showed that the microbial activity of the strain M17-1 was systematically higher than that of the strain M17-15 in all batches (Fig.S2 in Supplementary Materials). Therefore, in comparison with strain M17-15, strain M17-1 had a lower reduction rate for dissolved As(V).

As shown above, both strains M17-1 and M17-15 had the \( \text{arsC} \) gene. The \( \text{arsC} \) gene encodes the enzyme for As(V) reductase, which is responsible for the biotransformation of As(V) to As(III) (Krumova et al., 2008; Musingarimi et al., 2010). After As(V) reduction, the \( \text{arsB} \) gene acts as a specific efflux pump, which extrudes As(III) from the cytoplasm (Rosen, 2002; Silver and Phung, 2005). The very likely absence of \( \text{arsB} \), as observed in our isolates, probably indicated gene variations that decreased the homology of the primer sets. In this pathway, As(V) reduction would be a detoxification process, whereby the bacteria reduced As(V) to As(III), which would be more easily pumped out of the cell (Maity et al., 2011). Some studies have shown that \textit{Pseudomonas} sp. and \textit{Bacillus} sp. reduced As(V) under anaerobic conditions. They all belong to the facultative anaerobic bacteria (Wu et al., 2013).

The \( \text{arrA} \) gene was also observed in strains M17-15 and M17-1, which encodes for a reductase that catalyzes respiratory As(V) reduction (Saltikov and Newman, 2003; Afkar et al., 2003; Murphy and Saltikov, 2009). It may express in our study. Although two strains reduced 100% of As(V) at different initial As concentrations (0.5 and 7.5 mg/L), the reduction rates of As(V) by two strains were different. Some researchers showed that the reduction rate was dependent on initial As(V) concentrations, and incubation time (Maity et al., 2011). With the similar initial As(V) concentrations, and incubation time, less microbial activity and shorter reduction time indicated the relatively higher reduction rate of strain M17-15.

### 3.4. Bioreduction of solid phase As

#### 3.4.1. Reduction of As(V) in scorodite

Results showed that a small amount of As (0.5 mg/L) was dissolved into the solution from the scorodite in controls where the medium was free of strains (Fig. 3a). Both strains M17-15 and M17-1 promoted the release of As from scorodite. In the presence of strains, dissolved As increased significantly. At the incubation time of 10 d, dissolved As was around 14.5 mg/L with strain M17-1, which is lower than that with strain M17-15 (18.1 mg/L) (Fig. 3a).

In addition, As species were controlled by the presence of strains. In controls, only As(V) was detected in solutions, with As(III) < 0.2 μg/L. Dissolved As(V) was expected to originate from scorodite dissolution. Harvey et al. (2006) showed that concentration of dissolved As was around 450 μg/L at pH 6.0 at 22 °C, although incongruent dissolution occurred above pH 3. In experiments, As(III) concentration increased and reached the maximum at 4 and 2 d for strains M17-15 and M17-1 batches, respectively.
From scorodite. The reduction rate of As(V) by strain M17-15 is to As(III) would be the main reason for the enhanced release of As and/or solids in the presence of the strains. The reduction of As(V) though strain activity was inhibited by high pH. Krause (1989) the observation that As(V) concentration increased after 6 d even solutions (4 mg/L). The low Fe(III)/As(V) and high pH would explain evidenced by high total Fe (including dissolved and colloidal forms) in dissolved total As. The reason is that dissolved Fe was precipitated strain M17-15 (Fig. 3c). However, dissolved total Fe is far less than dissolved total As. The reason is that dissolved Fe was precipitated as Fe hydroxide colloids at high pH (around 9.5), which was evidenced by high total Fe (including dissolved and colloidal forms) in solutions (4 mg/L). The low Fe(III)/As(V) and high pH would explain the observation that As(V) concentration increased after 6 d even though strain activity was inhibited by high pH. Krause (1989) showed that the solubility of As in scorodite is related to the Fe/As and pH, with the higher solubility at the higher pH and lower Fe/As.

### 3.4.2. Reduction of goethite-adsorbed As(V)

The synthesized goethite had As content of 5.6 mg/g Fig. 4 shows change of dissolved As species in the batches. The adsorbed As(V) was desorbed in control batches without strains. The desorbed As reached around 2.1 mg/L at 2 d and exhibited a relatively stable concentration after 2 d (Fig. 4). The desorbed As accounted for 19.6% of total As adsorbed on goethite. O’Reilly et al. (2001) also found that after the initial rapid desorption, only a small amount of additional desorption occurred from goethite at longer time. Besides, a control with inactive cells showed that the dead cells did not evidently affect As(V) desorption (data not shown).

In the presence of strains, dissolved As concentration increased in the system of culture and As(V)-adsorbing goethite. After 20 d incubation, total dissolved As concentrations were 4.0 and 3.75 mg/L in batches with strains M17-15 and M17-1, respectively. If we subtracted the desorbed As obtained in controls, the released As by strain M17-15 accounted for 16.1% of total As on goethite, and by strain M17-1 13.8%. Therefore, strain M17-15 induced more As released from As(V)-adsorbing goethite than strain M17-1.

During 0–4 d incubation, only As(III) was detected with undetectable As(V) in solutions of batches with strains M17-15 and M17-1. It indicated that As(V) was reduced to As(III) by strains M17-15 and M17-1. After 4 d, both As(III) and As(V) concentrations increased gradually. The increase in As(III) concentration was the result of As(V) reduction by strains. However, the increase in As(V) concentration would be caused by the increase in solution pH due to desorption of As(V) from the surface of goethite at high pH. In this study, pH value increased from 8.4 to 9.5 during 6 d incubation (date not shown). In addition, As(III) re-oxidation by dissolved oxygen in solutions would lead to the increase in As(V) concentrations (DO around 6.0 mg/L) although the decrease in As(III) concentration was not detected. Grafe et al. (2001) showed that adsorption of As(V) on goethite was dependent on solution pH, with the lower adsorption at the higher pH. Both As(III) and As(V) concentrations kept relatively stable after 16 d, due to the limited strain activity. During the experiments, Fe(II) concentrations in solutions were below the detection limit in both batches (<0.1 mg/L), which indicated that goethite was not reduced in the presence of strains M17-15 or M17-1. Therefore, results of Fe species also supported that desorption of As(V) and/or reductive desorption of As(III) contributed to the release of As in the presence of the strains.

Arsenic species in goethite were determined by XANES, which are shown in Fig. 5. At 0 d incubation, no As(III) was detected, and As(V) was the only As species in goethite. In the presence of strain M17-15, As(III) accounted for 8% of total As at 4 d incubation, which increased to 21% after 16 d incubation (Fig. 5a). In comparison with strain M17-15, less As(III) was observed in goethite from the system in the presence of strain M17-1 (Fig. 5b). At 4 d incubation, As(III) accounted for 1% of total As, after which relative proportion of As(III) generally kept constant (around 6%). Considering dissolved As species and As species in goethite during incubation, the presence of strain M17-15 led to a higher As(III) content in goethite, and further released more As from goethite, relative to the presence of strain M17-1. Accordingly, it suggested that reduction of adsorbed As(V) should be the major cause of As release from goethite, in addition to desorption of As(V).

At 0–4 d, only As(III) was detected in solutions, concentration of which was generally equal to As desorbed from goethite. Therefore, As(V) reduction was expected to occur in solution at this period. After 4 d incubation, As(III) was detected on the surface of goethite, and As(III) content in goethite generally increased with the increase in incubation time (Fig. 5). Arsenic(III) content of goethite increased at 4–16 d in the presence of strain M17-15, while between 4 and 12 d in the presence of strain M17-1. Increases in As(III) concentrations in both solution and goethite indicated that As(V) reduction may directly occur on the surface of goethite. After reduction of adsorbed As(V) to As(III), As(III) was partly released into solution and partly retained on goethite. After that, both dissolved As(III) and As(V) kept relative constant. Due to the limited activity of strains, relative proportion of As(V) and As(III) in goethite kept stable after 16 d in the presence of strain M17-15; and after 12 d in the presence of strain M17-1.

### 3.4.3. Release and transformation of sedimentary As

Fig. 6 shows change of dissolved As concentration in batches with sediment M15. In controls without strains, As release was observed from the sediment at the first 2 d. After that, concentration of dissolved As showed relatively constant, which kept at around 125 μg/L (Fig. 6). However, strains M17-15 and M17-1 promoted the release of As from the sediment into solutions. With the strains, higher As concentration was observed at the first
2 d than that in controls. After 2 d incubation, dissolved As concentration gently increased with the incubation time in batches with strains (Fig. 6). Dissolved As concentrations were 200 and 175 mg/L with strains M17-15 and M17-1, respectively, at 6 d. It indicated that As was released from the sediment with the presence of strains M17-15 and M17-1. Since solution pH kept relatively constant during experiments (7.0 ± 0.2), As desorption due to pH variation would not be the cause for the increase in As concentration in the incubation batches. Furthermore, dissolved As concentration is higher in the batches with strain M17-15 than in the batches with strain M17-1. It demonstrated that strain M17-15 promoted As release from the sediment more apparently than strain M17-1, which is consistent with As release from scorodite and As(V)-loading goethite. Since clay minerals (64%) are the major phases in M14, followed by quartz (20%), calcite (12%), feldspar (2%) and dolomite (1%), As would be mainly fixed to clay minerals and calcite. Therefore, As was expected to be released from clay minerals and/or calcite in M14 in the presence of the strains. In controls, it was found that As(V) was the only As species in solutions. It may indicate that As(V) was desorbed from the sediment after sterilization. However, only As(III) was detected in solutions of batches with strains (Fig.S3 in Supplementary Materials), although DO content was around 5.0 mg/L in suspensions. XANES spectra showed that As predominantly occurred as As(V) in the pristine sediment (M14) (Fig.S4 in Supplementary Materials). Therefore, both strains M17-15 and M17-1 led to As(V) reduction to As(III). Two possible pathways were expected for the release of As from the sediment in the presence of the strains. One is that As(V) in the sediment was firstly released into solution and then reduced to As(III) with the presence of the strains. The other is that the strain firstly reduced sediment As(V) to As(III) in-situ, and then the produced As(III) on the surface of sediment was released into solution due to low affinity of As(III) species to mineral surfaces (Guo et al., 2007; Manning et al., 1998). Due to the absence of dissolved As(V), the plausible pathway included reduction of sediment As(V) and As(III) mobilization, which is also supported by the data from experiments with scorodite and As(V)-loading goethite. Mirza et al. (2014) also observed As(V) reduction and mobilization from sediments by dissimilatory arsenate-respiring bacteria. During the incubation, the As released in the presence of strains M17–15 and M17–1 accounted for 17.6% and 12% of total As in the sediment, respectively. This As fraction would be the exchangeable and/or adsorbed As in the sediment. At the end of incubations, dissolved As concentration generally kept constant, demonstrating that the release of As was not obvious due to the limited bacterial activity (2.0−3.5 nmol/L ATP). It showed that the activity of bacteria had a significant effect on the release of As from the sediment (Xie et al., 2011).

3.5. Environmental implication

Arsenic(V)-reducing bacteria are of significance in controlling As cycling in groundwater environment. Our previous study showed that anaerobic bacteria, including NO3−-reducing bacteria Pseudomonas sp., Fe(III)-reducing, SO42−-reducing and As(V)-reducing bacteria, were widely present in the aquifer sediments from the Hetao basin (Li et al., 2014). In addition to anaerobic As(V) reducing bacteria, which led to As mobilization due to the higher mobility of As(III) than As(V) (Li et al., 2014), anaerobic Fe-respiring bacteria

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**Fig. 5.** Arsenic K-edge XANES spectra of goethite in batches with the presence of strains M17-15 (a) and M17-1 (b).

**Fig. 6.** Variation in dissolved As concentration with the incubation time in the system of culture and sediment (M14).
(IRB) would stimulate the reductive dissolution of Fe(III) oxides/oxhydroxides, and therefore the release of As (Guo et al., 2008b; Kar et al., 2010; Selim et al., 2010a; Rowland et al., 2009). This study showed that aerobic As(V)-reducing bacteria, *Pseudomonas stutzeri* strain and *Bacillus flexus* strain, also led to As(V) reduction and subsequently As release from As(V)-adsorbing minerals, As(V)-containing crystalline, and natural sediments in oxidizing conditions. Bachate et al. (2009) showed that As(V)-reducing bacteria potentially mediated As transformations in soils, and their role in As cycling may become relevant with changing environmental conditions.

Two microbial pathways were related to As(V) reduction to As(III). One is As(V) respiration reduction, which is associated with *arr* genes. Arsenic(V)-respiring genes have been found in *Geobacter* (Reyes et al., 2008), *Shewanella* (Murphy and Saltikov, 2009), and *Clostridium* (Mirza et al., 2014). Munford et al. (2012) concluded that the solubility and transport of As in shallow groundwater was influenced by microbial respiratory reduction of As(V) to As(III). The other is As(V) detoxification, which is related to *ars* genes. It has observed that *Escherichia coli* (Wu and Rosen, 1993), *Pseudomonas* (Cai et al., 1998), *Acidithiobacillus* (Butcher et al., 2006), *Corynebacterium* (Ordóñez et al., 2005), *Staphylococcus* (Lin et al., 2007), and *Geobacter metallireducens* strain NRC-1 (Wang et al., 2004) had the *ars* genes. As shown in strains M17–15 and M17-1 in this study, *Shewanella* sp. strain ANA-3 also had both detoxification *ars* genes and As(V)-respiring *arr* genes. Although several environmental conditions affected the expression of respiration *arr* and detoxification *ars* As(V) reductase genes (Saltikov et al., 2005), As(V)-reducing microbes would regulate As transformation and migration in groundwater systems.

No Fe(III) reduction was observed in this study, although the investigated As(V)-reducing bacteria caused As mobilization from the sediment via As(V) reduction. Accordingly, they led to the increase in dissolved As concentration, but did not affect dissolved Fe concentration. It may be used to explain the fact that dissolved As was decoupled with dissolved Fe in most high As groundwaters (Selim et al., 2010a,b; Lu et al., 2011; Xie et al., 2012; Guo et al., 2011a, 2014b).

After reducing As(V) in the solids, those strains would result in the release of As(III) from the solids in oxidic conditions. Therefore, As(III) may be observed in oxic aquifers in the presence of As(V)-reducing bacteria. Some studies have reported a relative As(III) abundance to total As in groundwaters in oxidizing conditions (Abdullah et al., 1995; O’Reilly et al., 2010). The ratio of As(III) to As(V) can vary greatly as a result of variations in the abundance of redox-active solids, especially the activity of microorganisms (Smedley and Kinniburgh, 2002). Therefore, aerobic As(V)-reducing bacteria are the plausible causes for the fact that As(III) occurred in facultative oxic aquifers.

4. Conclusions

Two strains, isolated from the aquifer sediment with high As groundwater from the Hetao basin, included *Bacillus* sp. M17–15 and *Pseudomonas* sp. M17–1. Strains M17–15 and M17-1 grew in culture media with high As concentrations under oxidic conditions. They had a good resistance to both As(III) and As(V). The strains had As(V)-respiring *arr* and As(V) detoxification *ars* genes, and led to reduction of dissolved As(V), adsorbed As(V), crystalline As(V) and sediment As(V) to As(III). The reduction rate of solid As(V) was lower than dissolved As(V) in the presence of the strains. The reduction promoted the release of solid As into the strains. In comparison with strain M17–1, strain M17-15 had the higher As(V) reduction rate and therefore the more apparent release of As from solids into solutions. It was suggested that the indigenous microorganisms would affect As transformation in groundwater systems, and play an important role in the formation of high As groundwater.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.03.034.

References


