The fate of arsenic adsorbed on iron oxides in the presence of arsenite-oxidizing bacteria

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HIGHLIGHTS

- Microbial cells lowered total As adsorption by minerals.
- Microbial oxidation proceeded much slowly in solid phase than in aqueous phase.
- Minerals with a higher affinity for As(III), hindered As(III) oxidation more.
- Under aerobic conditions, As(V) is the primary form in solid and aqueous phases.

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ABSTRACT

Arsenic (As) is a redox-active metalloid whose toxicity and mobility in soil depend on its oxidation state. Arsenite [As(III)] can be oxidized by microbes and adsorbed by minerals in the soil. However, the combined effects of these abiotic and biotic processes are not well understood. In this study, the fate of arsenic in the presence of an isolated As(III)-oxidizing bacterium (*Pseudomonas* sp. HN-1, 10^9 colony-forming units (CFUs) $\cdot$ ml⁻¹) and three iron oxides (goethite, hematite, and magnetite at 1.6 g L⁻¹) was determined using batch experiments. The total As adsorption by iron oxides was lower with bacteria present and was higher with iron oxides alone. The total As adsorption decreased by 78.6%, 36.0% and 79.7% for goethite, hematite and magnetite, respectively, due to the presence of bacteria. As(III) adsorbed on iron oxides could also be oxidized by *Pseudomonas* sp. HN-1, but the oxidation rate (1.3 μ mol h⁻¹) was much slower than the rate in the aqueous phase (96.2 μ mol h⁻¹). Therefore, the results of other studies with minerals only might overestimate the adsorptive capacity of solids in natural systems; the presence of minerals might hinder As(III) oxidation by microbes. Under aerobic conditions, in the presence of iron oxides and As(III)-oxidizing bacteria, arsenic is adsorbed onto iron oxides within the adsorption capacity, and As(V) is the primary form in the solid and aqueous phases.

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1. Introduction

The elevated and extensive contamination of arsenic (As) in soil and groundwater causes serious health risks in many regions around the globe (Smith et al., 1998; Smedley and Kinniburgh, 2002). The toxicity and mobility of As in soil depend on its chemical speciation. Inorganic forms are more toxic than organic forms, and arsenite(As(III)) is generally regarded as more mobile and more

toxic than arsenate (As(V)) (WorldHealthOrganization, 1981; Liu et al., 2001). The environmental and toxicological effects of As are related to the valence states and distribution between the immobile and mobile forms (Cullen and Reimer, 1989; Langner and Inskeep, 2000). Therefore, As oxidation and distribution in soil affect its remediation and human health risk assessment. Arsenic oxidation is kinetically slow with atmospheric oxygen; therefore, it is believed that soil components, including minerals and microbes, contribute to As(III) oxidation in oxic soil (Eary and Janet, 1990).

Microbial oxidation is an important factor that influences the fate of As in soil. More than 30 strains representing at least nine genera have been reported, including α -, β -, γ -*Proteobacteria*. They

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include chemoautotrophic As(III) oxidizers and heterotrophic As(III) oxidizers (Oremland and Stolz, 2003). For chemoautotrophic As(III) oxidizers, the oxidation of As(III) generates energy, which promotes growth and proliferation (Santini et al., 2000). For heterotrophic As(III) oxidizers, the oxidation of As(III) is described as a detoxification mechanism catalyzed by the periplasmic enzyme arsenite oxidase (Muller et al., 2003). Diverse As(III) oxidation bacteria have not only been found in As-contaminated soils (Cai et al., 2009), but also in low As-containing soil (Bahar et al., 2012).

Soil minerals, especially iron(hydr) oxides, are considered important solid phases, which are responsible for As mobilization or immobilization in soils and sediments (Daus et al., 1998; Frommer et al., 2011). Goethite, hematite and magnetite, which are widespread in soils (Schwertmann and Cornell, 1991), have been found to be effective adsorbents of As(III) and As(V) (Arai et al., 2004; Ona-Nguema et al., 2005; Lakshminathiraj et al., 2006; Gimenez et al., 2007; Ohe et al., 2010). Many studies were carried out to study the characteristics and mechanisms of As adsorption onto goethite, hematite and magnetite (Ona-Nguema et al., 2005; Gimenez et al., 2007). Gimenez et al. observed the highest As(III) sorption on the hematite surface over the entire pH range compared to goethite and magnetite (Gimenez et al., 2007). Iron(hydr) oxides could only slightly oxidize As(III), when they were induced or catalyzed, for example, by Fe(II) or light. Early studies showed the rapid oxidation of As(III) to As(V) at the surface of goethite, magnetite and ferrihydrite in Fe(II)-mediated reactions (Amstaetter et al., 2010; Ona-Nguema et al., 2010). It is reported that goethite interacting with dissolved Fe(II) produced the highest redox activity compared with other Fe(III) (oxy)hydroxides (Elsner et al., 2004). Photoinduced oxidation of As(III) by goethite has also been reported and can be significantly restricted by humic acid (Bhandari et al., 2012; Wang et al., 2013).

Minerals and microbes in soils influence As by adsorption, oxidation or reduction; thus, the interaction between these abiotic and biotic processes must be determined. The combined effects of bacteria and Mn oxides were investigated. It was shown that As(III) was oxidized most rapidly in a mixed cell- MnO₂ system, although the total sorption of As on minerals was lower (Jones et al., 2012). The microbial reduction of As(V) in the presence of iron oxides was also analyzed. Huang et al. suggested that the attachment of microbial cells to mineral surfaces promoted As(V) desorption, thereby facilitating As(V) reduction (Huang et al., 2011b). From a different perspective, Langner et al. indicated that sorbed As(V) could only be slightly reduced. Therefore, the desorption of As(V) was too slow to cause an increase in the aqueous content (Langner and Inskeep, 2000). Above all, the combined oxidation of bacteria and Mn oxides, and microbial reduction in the presence of iron oxides have been reported in earlier studies. However, with the coexistence of iron(hydr)oxides and As(III)-oxidizing bacteria in soil, the As oxidation and adsorption processes remain poorly understood.

In the present study, the mixed cell-iron oxide batch experiments were used, and the objectives of the study were to investigate (1) the adsorption by iron oxides in the presence of As(III)-oxidizing bacteria, (2) the As(III) oxidation by As(III)-oxidizing soil bacteria in the presence of iron oxides (goethite, hematite and magnetite) and (3) the distribution of As(III) and As(V) between solution and iron oxides after treatment with As(III)-oxidizing soil bacteria.

2. Materials and methods

2.1. Iron oxides synthesis and characterization

The experiments were conducted with three iron oxides:

goethite, hematite and magnetite. Goethite and hematite were synthesized by the method of Schwertmann and Cornell (Schwertmann and Cornell, 1991). Magnetite was synthesized by the method of Jolivet and Tronc (Jolivet and Tronc, 1988). These iron oxides were washed repeatedly with double-distilled water until they were free of excess salt; then, they were freeze-dried. The iron oxides were identified by X-ray diffraction (XRD) using an X-ray powder diffractometer (X'Pert PRO MPD diffractometer) with Cu K α radiation and scanned from 10° to 80° 2 θ with a continuous scan rate of 0.025°/18 s. The surface area of goethite, hematite and magnetite was 36.8 $\pm$ 1.3, 22.0 $\pm$ 0.3, and 67.5 $\pm$ 0.3 m² g⁻¹, respectively, based on 5-point N₂-BET measurements (Micromeritics Gemini V 2.0, U.S.).

2.2. The isolation and identification of As(III)-oxidizing bacteria

A soil sample ([As] = 284 mg kg⁻¹) collected from mine of Zhuzhou, Hunan, China, was used as an inoculum for enrichment culture. The isolation and subsequent growth experiments were carried out with a modified chemically defined medium (CDM) (8.12 mM MgSO₄, 18.7 mM NH₄Cl, 7 mM Na₂SO₄, 0.0574 mM K₂HPO₄, 0.457 mM CaCl₂, 44.6 mM Na-lactate and 9.5 mM NaHCO₃, with the pH adjusted to 7.0) as described by Weeger et al. (1999). As-resistant bacteria were enriched with 1.33 mM As(III) under oxic incubation. After enrichment, samples were serially diluted, and a cell suspension was spread onto CDM plates with 1.33 mM As(III) to obtain single colonies. A pure culture was obtained by successive isolation of colonies in As(III)-supplemented medium. The ability of the obtained As-resistant bacteria to oxidize As(III) was tested using a qualitative AgNO₃ screening method as described by Liao et al. (2011).

Bacterial genomic DNA of the obtained As(III)-oxidizing bacterium was extracted using TIANamp Bacteria DNA Kit (TIANGEN, China) following the manufacturer's instructions. Then, the bacterial 16S rDNA gene was amplified using universal primers, 27f and 1492r (Lane, 1991). Cells in late exponential growth phase were inoculated in CDM in subsequent batch experiments.

2.3. Batch oxidation and adsorption experiments

2.3.1. Arsenic adsorption in the presence of bacteria

Arsenic adsorption by iron oxides (goethite, hematite and magnetite) in the presence of bacteria was studied. The experimental group is named the cell-iron oxide group in the following sections. Abiotic control experiments of iron oxides only were also conducted, which is named the mineral-only group in the following sections.

Experiments were carried out in triplicate using sterile Erlenmeyer flasks with 100 ml CDM. The reaction was initiated by adding As(III) (NaAsO₂) (600 μ M), cell cultures (10⁹ CFU ml⁻¹) and iron oxides (1.6 g L⁻¹), with a constant ionic strength (0.1 M NaCl). The initial As concentration was chosen to reach adsorption saturation. The flasks were shaken at 25 °C at 150 rpm for 24 h. During the experiments, 2 ml samples were removed periodically from the flasks and passed through a 0.22- μ m filter. The concentrations of total dissolved As were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, OPTIMA 8300, U.S.). Then, the amount of adsorbed As was calculated by subtracting the dissolved As from the total As.

2.3.2. Effect of contact order of bacteria and goethite on the oxidation and adsorption of As

Three groups of cell-goethite batch experiments of different contact order of bacteria and goethite were used for the experiments: (1) bacteria, goethite and As(III) were added simultaneously

(abbreviated SBAG); (2) bacteria and As(III) were incubated for 1 h before goethite was added (abbreviated BAG); and (3) goethite and As(III) were incubated for 1 h before bacteria were added (abbreviated GAB).

Experiments were carried out in triplicate using sterile Erlenmeyer flasks with 100 ml CDM. The reaction was initiated by adding As(III) (NaAsO_2) (160 μM), cell cultures (10^9 CFU ml^{-1}) and goethite (1.6 g L^{-1}), with a constant ionic strength (0.1 M NaCl). The concentration of As(III) and goethite were chosen to ensure that the ratio of As to Fe ($\text{Fe/As} \approx 100$) after incubation was appropriate for the determination of the ratio of As(III) and As(V) by XANES analysis. In other studies on As(III) oxidation in the presence of iron oxides, the Fe/As ratio ranged from 5 to 580 (Ona-Nguema et al., 2005, 2010; Bhandari et al., 2011, 2012). The flasks were shaken at 25 °C at 150 rpm for 10 h. The adsorption reached equilibrium in 10 h. Abiotic control experiments of goethite only were also conducted. During the experiments, 2 ml samples were removed periodically from the flasks, passed through a 0.22- μm filter, and stored at –80 °C until As speciation analysis. At the end of the reaction, the incubation suspensions were centrifuged, washed twice and then freeze dried for arsenic XANES analysis.

2.3.3. Comparison of As(III) oxidation in three cell-iron oxide systems

Batch experiments for microbial oxidation in the presence of three iron oxides were conducted to determine the apparent As(III) oxidation and the proportion of As(III) to As(V) in the solid phase. Three mixed cell-iron oxide batch experiments were conducted using *Pseudomonas* sp. HN-1 with hematite, goethite (SBAG group) and magnetite, separately. Abiotic control experiments of iron oxides only were also conducted.

Experiments were carried out under the same conditions as those given in 2.3.2.

2.4. Arsenic speciation analysis

Arsenic speciation was determined by HPLC-ICP-MS (7500a; Agilent Technologies) as described in the literature (Zhu et al., 2008). Chromatographic columns were obtained from Hamilton and consisted of a precolumn (11.2 mm, 12–20 μm) and a PRP-X100 10- μm anion-exchange column (250 $\times$ 4.1 mm). The mobile phase consisted of 10 mM diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) and 10 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using nitric acid. The mobile phase was pumped through the column isocratically at 1 ml min^{-1} . Arsenic species in the samples were identified by comparing their retention time with those of the standards, including arsenite (As(III)) and arsenate (As(V)), and quantified by external calibration curves with peak areas.

2.5. Arsenic XANES (K-edge) analysis

The As K-edge XANES spectra of the solid samples were collected on beamline 1W2B at the Beijing Synchrotron Radiation Facility (BSRF), China. The electron beam energy was 2.5 GeV, with a maximum beam current of 250 mA. Arsenic K-edge XANES spectra were collected at room temperature in the fluorescence mode for the samples and transmission mode for the reference materials. Data analysis was conducted using Athena software. Every sample was normalized, and linear combination fitting (LCF) was then used to identify the As species. Reference materials included As(III)-adsorbed goethite, As(V)-adsorbed goethite (Ona-Nguema et al., 2010), arsenite and arsenate. The accuracy of the As(III)/ ΣAs ratio is $\pm 2\%$, and components contributing less than 5% are not statistically significant (Morin et al., 2003).

2.6. FESEM analysis

Iron oxides (goethite, hematite, and magnetite) and batch reaction products, including mixed cell-goethite, cell-hematite, and cell-magnetite samples were imaged by field-emission scanning electron microscopy (FESEM). Samples of batch reaction products were centrifuged and fixed with 2.5% glutaraldehyde at 4 °C. Then, they were washed with phosphate buffer three times. Samples were dehydrated with alcohol, and dried with liquid CO_2 using the critical point method. Finally, samples were imaged using a Hitachi FESEM with a 3 kV accelerating voltage and working distances of 8.3–8.7 mm.

3. Results and discussion

3.1. Isolation and identification of *Pseudomonas* sp. HN-1

An aerobic heterotrophic bacterial strain was isolated. BLAST and similarity analysis of the near full length 16S rRNA gene sequences (1,465 bp) of the As(III)-oxidizing bacterium showed that it belonged to *Pseudomonas* (99% similarity, GeneBank accession number: KJ452337). The bacterial strain was named as *Pseudomonas* sp. HN-1. The strain cannot grow in media designed for chemolithotrophic metabolism using As(III) as the sole electron donor and it requires an organic carbon source (e.g., sodium lactate) for growth. With the result that As(III) oxidation corresponded with microbial growth (data not shown), it is suggested that the mechanism of As(III) oxidation by the isolate was related to As detoxification rather than energy generation (Oremland and Stolz, 2005).

3.2. Arsenic adsorption in the presence of bacteria

Arsenic adsorption by hematite, goethite, and magnetite with or without As(III)-oxidizing bacteria during the incubation is shown in Fig. 1. In the cell-iron oxide groups and the mineral-only groups, the maximum As adsorption by hematite was higher than that by goethite and magnetite. The abiotic mineral studies of Gimenez et al. showed higher As adsorption by natural hematite than natural goethite and magnetite (Gimenez et al., 2007). It is reported that As(III) forms different inner-sphere surface complexes for hematite and goethite (Ona-Nguema et al., 2005).

In our study, for the cell-iron oxide group, after 10 h of incubation, the adsorption amount of As was 34.4, 255.3, and 37.3 $\mu\text{mol g}^{-1}$ by goethite, hematite and magnetite, respectively. However, for the mineral-only group, the adsorption amount of As was 160.4, 398.6 and 183.6 $\mu\text{mol g}^{-1}$ by these three minerals, respectively. Total As adsorption decreased by 78.6%, 36.0% and 79.7% for these three cell-iron oxide groups compared with corresponding mineral-only groups, respectively (Fig. 1). A lower amount of As was adsorbed by cell-iron oxide batch experiments than by minerals alone. The reduced adsorption may result from mineral surface passivation by extracellular polymeric substances (EPS), which contain phosphate and carboxylate groups that could compete with As for adsorption sites (Huang et al., 2011a). It was reported that the adsorption of As(V) and As(III) strongly decreased with increasing addition of EPS for ferrihydrite, goethite and hematite (Huang et al., 2011a). EPS play an important role during the adhesion of bacteria to minerals. Covalent bond formations during the adsorption of EPS from bacteria and Fe-oxy(hydr)oxides have been demonstrated (Omoike and Chorover, 2006; Fang et al., 2012). However, the functions of EPS for different minerals are not always the same. In the absence of EPS, the adhesion of bacteria to clay minerals was reduced, but adhesion to goethite was enhanced (Hong et al., 2013).

The images of mixed cell-iron oxide after the batch experiments

Fig. 1. Adsorbed As by (a) goethite, (b) hematite and (c) magnetite during the incubation with or without bacteria.

are shown in Fig. 2. The macroscopic results showed the attachment between bacteria and minerals. The adhesion of bacteria to minerals might block the adsorption sites of As on the minerals. Therefore, As adsorption might be impeded by bacteria due to occupation of adsorption sites. In addition, no morphological change in the iron oxides was observed after incubation with As and bacteria, which is similar to the observation in cell-Mn oxide oxidation by Jones et al. (Jones et al., 2012). However, different morphologies were detected by Muehe et al. during microbial Fe(III) reduction, as revealed by the formation of secondary Fe mineral products (Muehe et al., 2013). In the study of Jones et al., it was observed that more As was adsorbed by δ -MnO₂ alone than by the cell- δ -MnO₂ experiments (Jones et al., 2012). It was proved that microbial cells and EPS could mobilize As from Fe(III)-hydroxides because of competitive interactions between adsorbed As and functional moieties of cell surface molecules and EPS at mineral surfaces (Huang et al., 2011a). Adsorption could be reduced due to surface passivation, as well as a decrease in oxidation and reduction by minerals. Parikh et al. indicated that the reaction rates were reduced when bacterial adhesion strongly passivated δ -MnO₂ and reduced the As(III) interactions with the mineral surface (Parikh et al., 2010). It is proposed that As(V) adsorption could inhibit As(III) oxidation by blocking adsorption sites (Zhu et al., 2009).

Early studies indicated that the presence of organic matter (ions in solution) also influenced As(III) adsorption or oxidation by δ -MnO₂ by blocking reaction sites (Zhu et al., 2009; Parikh et al., 2010). Iron oxides and Mn oxides, as common soil minerals (Daus et al., 1998; Post, 1999), showed a similar tendency of adsorption in the presence of bacteria, which can partially reflect real conditions in soil. Therefore, mineral-only experiments might overestimate As adsorption by minerals in soils.

3.3. Effect of contact order of bacteria and goethite on oxidation and adsorption of As

Fig. S1 summarizes the As content in the aqueous phase at the end of incubation. The adsorption percentage of the control, SBAG, BAG and GAB was 99.97%, 96.98%, 96.26% and 97.23%, respectively. The amount of As adsorbed by goethite decreased in the following order: control > SBAG > BAG. The decrease of As adsorption was related to the exposure time of bacteria, providing additional evidence of the inhibiting effect of bacteria on As adsorption by minerals.

Synchrotron-based measurement of As speciation by XANES confirmed that most of the As was found in the solid phase in the form of As(V) after incubation (Fig. 3). The ratio of As(III) to total As after incubation decreased in the following order: GAB (27%) > SBAG (6.1%) > BAG (4.7%) (Table 1). For group BAG, after a 1-h incubation of bacteria and As, most of As(III) was oxidized to As(V), and then adsorption occurred (Fig. 4(b)). However, for group GAB, after a 1-h incubation of goethite and As(III), most of the As(III) was adsorbed on the mineral surface, and then oxidation occurred there (Fig. 4(c)). Thus, the oxidation of As(III) primarily occurred in the aqueous phase for group BAG and in the solid phase for group GAB. Based on these results, 73% of As(III) in solid phase was oxidized in 9 h ($1.3 \mu\text{mol h}^{-1}$). The calculated oxidation rate was both around $1.3 \mu\text{mol h}^{-1}$ according to the proportion of As(V) in solid phase at 8 h and 10 h, which is 65.7% and 82.4%, respectively. However, in the absence of minerals, As(III) oxidation could be almost completed in 10 min in the aqueous phase ($96.2 \mu\text{mol h}^{-1}$) (Fig. S2). Therefore, the oxidation rate is approximately 75 times faster in the aqueous phase than in solid phase by the As(III)-oxidizing bacterium, *Pseudomonas* sp. HN-1, indicating that the presence of minerals hindered microbial oxidation. For group SBAG, the proportion of As(III)/As(V) after incubation was closer to that of BAG than GAB.

Fig. 2. Field emission scanning electron microscopy (FESEM) images of (a) goethite alone, (b) mixtures of cells-goethite, (c) hematite alone, (d) mixtures of cells-hematite, (e) magnetite alone, (f) mixtures of cells-magnetite.

In another word, the oxidation and adsorption process in group SBAG was similar to the process in group BAG. Therefore, we suppose that As(III) oxidation took place faster than As(III) adsorption at this concentration of As(III) ($160 \mu\text{M}$) and cells (10^9 CFU ml^{-1}). In the case of microbial reduction, Huang et al. and Ohtsuka et al. indicated that the reduction of adsorbed As(V) is much slower than that of dissolved As(V) by As(V)-reducing bacteria (Huang et al., 2011b; Ohtsuka et al., 2013). They demonstrated that the presence of minerals hindered microbial As(V) reduction. In the former study, a strong decrease in As(V) reduction rate was found as the solid concentration and the extent of As(V) sorption increased. The reduction rate was 10–78 times faster in the aqueous phase than in solid phase based on different amounts of solid. In the latter study, the reduction rate was approximately 10 times faster in the

aqueous phase than in the solid phase. Furthermore, Ohtsuka et al. has shown arsenic release from paddy soil by an arsenate-reducing bacterium *Geobacter* sp. OR-1, due to the direct As(V) reduction in the solid phase. However, an As(V)-reducing bacteria, *Clostridium* sp. CN8, was observed to have the ability to only reduce As(V) in the aqueous phase, and could hardly reduce sorbed-As. A slight desorption of As from Fe(hydr)oxide was found by strain CN8 (Langner and Inskeep, 2000). These studies concentrated on As-iron oxide-As(V)-reducing bacteria systems. However, our studies focused on As-iron oxide-As(III)-oxidizing bacteria systems. To the best of our knowledge, no investigation of the As fate in the presence of As(III)-oxidizing bacteria and iron oxides has been reported.

Fig. 3. Arsenic K-edge XANES data for As(III) and As(V) adsorbed onto goethite at the end of incubation (As(III)/As(V): reference materials; control: mineral-only experiments without bacteria; SBAG: bacteria, goethite and As(III) added simultaneously; BAG: bacteria added before goethite; GAB: goethite added before bacteria.).

Table 1

Linear combination fitting of As K edge XANES spectra for the samples.

Std	Percentage, %				
	SBAG(goethite)	BAG	GAB	Hematite	Magnetite
As(III)	6.1	4.7	27.0	10.9	—
As(V)	93.9	95.3	73.0	89.1	100

3.4. Comparison of As(III) oxidation in three cell-iron oxide systems

Three mixed cell-iron oxide batch experiments were conducted using As(III)-oxidizing bacteria with hematite, goethite and magnetite, separately. The amount of As in the aqueous phase after 10 h of incubation is shown in Fig. S3. In the presence of As(III)-oxidizing bacteria, As(V) was the predominant species in the aqueous phase. As speciation on iron oxides was determined with XANES analysis. The XANES results revealed that As(III) was the only species on the iron oxides in the abiotic control experiments (data not shown). As(V) was the predominant species on iron oxides in the cell-iron oxide experimental group (Fig. 5). The ratio of As(III) to total As after incubation decreased in the following order: hematite (10.9%) > goethite (6.1%) > magnetite (0) (Table 1). Based on the analysis of As concentration in aqueous solution, the results indicated that with the maximal adsorption capacity, less As(V) was observed on the surface of hematite than that on the surface of goethite and magnetite at the end of incubation. This result provides additional evidence of a faster oxidation rate in the aqueous phase than in the solid phase by *Pseudomonas* sp. HN-1. Another possible reason of this result is that arsenic forms different structures with the iron oxides. It is reported that As(III) forms different inner-sphere surface complex on hematite and goethite (Ona-Nguema et al., 2005), which might made adsorbed As(III) on hematite a little more difficult to be oxidized.

According to mechanisms of As(V) reduction in solid phase (Huang et al., 2011b; Ohtsuka et al., 2013), there might be two explanations of As(III) oxidation in solid phase: one is directly oxidation of As(III) in solid phase; one is that the attachment of cells promoted As(III) desorption, and the desorbed As(III) was oxidized followed by adsorption of As(V) on the solid phase (Dong et al.,

(a)

(b)

(c)

Fig. 4. Concentration of dissolved As(III) and As(V) during incubation of (a) SBAG, (b) BAG and (c) GAB.

Fig. 5. Arsenic K-edge XANES data for As(III) and As(V) adsorbed onto hematite, goethite and magnetite at the end of incubation.

2014). So far, only oxygen, nitrate or chlorate reduction have been shown to be coupled to As(III) oxidation in the chemolithotrophs (Oremland et al., 2009). And oxygen has been shown to be coupled to As(III) oxidation in the heterotrophs. As(III) serves as an electron donor via the As(III) oxidase (Slyemi and Bonnefoy, 2012). For heterotrophic As(III) oxidizers, the oxidation of As(III) is described as a detoxification mechanism catalyzed by the periplasmic enzyme arsenite oxidase (Muller et al., 2003). Thus, As(III) was taken up into the periplasmic space, oxidized and released in the form of As(V) (Slyemi and Bonnefoy, 2012). The probability was greatly reduced for fixed As(III) to be taken up into periplasmic space, compared with free As(III). This might be one reason for the slower oxidation rate in the solid phase. The attachment of bacteria to minerals (Fig. 2) might reduce the contact between bacteria and As(III), thus reducing the oxidation of fixed As(III). As for the second explanation of As(III) oxidation in solid phase, the oxidation rate might be limited by low desorption concentration of As(III) (Huang et al., 2011b).

Therefore, minerals with a higher affinity for As(III) might offer greater obstacle for As(III) oxidation in soils. Parikh et al. demonstrated that various mineral surfaces could block Mn-oxide reaction sites, thus inhibiting As(III) oxidation (Zhu et al., 2009). Ehlert et al. implied that As(III) oxidation by birnessite could be inhibited by ferrihydrite (Ehlert et al., 2014). Thus, the valence state of As in soil is related to the mineralogical soil type. Regarding the remediation or removal of As from wastewater, oxidation by bacteria is recommended before adsorption by adsorbents, because oxidation in solid phase is slow. The inhibition of As(III) oxidation was caused by minerals with no oxidizing ability. However, in the presence of minerals with oxidizing ability, facilitation of As oxidation was dominant instead of inhibition. In the study of Jones et al., The apparent rate of As(III) oxidation rate was greater for the combined batch experiments in which bacteria and δ -MnO₂ were oxidizing As(III) at the same time than for either component alone (Jones et al., 2012).

4. Conclusion

In the presence of As(III)-oxidizing bacteria and iron oxides, As(V) is the primary form of inorganic As in the solid and aqueous phases under aerobic conditions. In addition, we found that the interaction between *Pseudomonas* sp. HN-1 cells and mineral

sorbents lowered the total As adsorption by minerals. Our results demonstrated that microbial oxidation proceeded much more slowly in solid phase than in the aqueous phase. The adsorption of As(III) onto mineral surfaces strongly reduced the rate of As(III) oxidation. Furthermore, this study showed that minerals with a higher affinity for As(III) offered greater obstacle for As(III) oxidation in soils. Therefore, the valence state of As in soil is related to the mineralogical soil type.

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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.chemosphere.2016.02.065>.

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