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Effect of microbial mediated iron plaque reduction on arsenic mobility in paddy soil

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Abstract

The potential of microbial mediated iron plaque reduction, and associated arsenic (As) mobility were examined by iron reducing bacteria enriched from As contaminated paddy soil. To our knowledge, this is the first time to report the impact of microbial iron plaque reduction on As mobility. Iron reduction occurred during the inoculation of iron reducing enrichment culture in the treatments with iron plaque and ferrihydrite as the electron acceptors, respectively. The Fe(II) concentration with the treatment of anthraquinone-2, 6-disulfonic acid (AQDS) and iron reducing bacteria increased much faster than the control. Arsenic released from iron plaque with the iron reduction, and a significant correlation between Fe(II) and total As in culture was observed. However, compared with control, the increasing rate of As was inhibited by iron reducing bacteria especially in the presence of AQDS. In addition, the concentrations of As(III) and As(V) in abiotic treatments were higher than those in the biotic treatments at day 30. These results indicated that both microbial and chemical reductions of iron plaque caused As release from iron plaque to aqueous phase, however, microbial iron reduction induced the formation of more crystalline iron minerals, leading to As sequestration. In addition, the presence of AQDS in solution can accelerate the iron reduction, the As release from iron plaque and subsequently the As retention in the crystalline iron mineral. Thus, our results suggested that it is possible to remediate As contaminated soils by utilizing iron reducing bacteria and AQDS.

Key words: iron reducing bacteria; iron plaque; arsenic; anthraquinone-2,6-disulfonic acid (AQDS); paddy soil; ferrihydrite
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Introduction

Arsenic (As) is a toxic and carcinogenic element, and is widely distributed in various environments, especially in the groundwater in Southeast Asia, where wellwater is widely used for irrigating rice fields (Nickson *et al*., 1998). Intensive use of As-contaminated groundwater for irrigation can cause the increasing level of As in paddy soils and As accumulation in rice grain (Meharg and Rahman, 2003; Williams *et al*., 2006). Moreover, mining activities also result in the As contamination in paddy soils around mining areas and thus lead to an As build-up in rice grain (Zhu *et al*., 2008a). For populations living on subsistence rice diets, the As contamination of rice grain contributes greatly to dietary As exposure (Zhu *et al*., 2008b). Rice is the staple food for around 50% of the world's population (Meharg *et al*., 2009). Therefore, chronic consumption As-contaminated rice will pose risks to human health.

Liu *et al*. (2004a) have shown that As can be sequestered in iron plaque of root surface of plants, thus reducing As uptake into plant tissues. Iron plaque is a precipitate of reddish-brown Fe oxides and is ubiquitously formed on the roots of paddy rice (Liu *et al*., 2004a, 2004b). Iron plaque is formed by abiotic oxidation or iron oxidizing bacteria (Weiss *et al*., 2003), and its structure is characterized as amorphous or crystalline Fe(oxyhydr)oxides (Hansel *et al*., 2001), which serve as preferred substrates for iron reducing bacteria.

Iron reducing bacteria that can gain energy for growth by coupling the oxidation of organic compounds to the reduction of Fe(III)oxides (Lovley *et al*., 2004) have been successfully enriched from rhizosphere (King and Garey, 1999), where this kind of becteria can account for 12% of total bacteria cells (Weiss *et al*., 2003). Several studies have demonstrated that the microbial reduction of As-bearing Fe(III)(hydro)oxides result in a dissolution of the solid phase, and this could potentially mobilize As held within or sorbed on the surface of the iron oxides (Cummings *et al*., 1999; Benner *et al*., 2002; Rowland *et al*., 2007). However, in recent studies, it has been observed that the Fe(III) reduction is also likely to form secondary iron phases which have a potential to sorb As (Kocar *et al*., 2006; Tufano *et al*., 2008). Thus, the effect of microbial iron reduction on As mobility is still a matter of debate.

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Although it is known that iron reducing bacteria colonized in the root surface, so far it is still unclear whether iron reducing bacteria can reduce iron plaque and thus lead to As release from iron plaque.

Therefore, an enrichment culture of iron reducing bacteria was set up to investigate whether iron reducing bacteria can reduce iron plaque and whether this reductive process can enhance or limit the As mobility.

1 Materials and methods

1.1 Soil samples

Soil samples were collected from a mining area of Bailutang in Shizhuyuan (25°48′N, 113°02′E) located in Chenzhou, Hunan Province, China. The soil sample was kept in PVC bottles and submerged with water under the field conditions, then transported to the laboratory. Soil pH (0.01 mol/L $CaCl_2$) was 6.10. The soil had the following chemical properties on a dry weight basis (mg/kg): totals P 396.9; total Fe 22410.2; and total As 42.

1.2 Enrichment culture experiment

An anoxic carbonate-buffered freshwater medium was used for enrichment of iron reducing bacteria in the As contaminated paddy soil. The basal medium contained (g/L): NaCl 1, $MgCl_2{\cdot}6H_2O$ 0.4, $CaCl_2{\cdot}2H_2O$ 0.1, NH_4Cl 0.25, KH_2PO_4 0.2, KCl 0.5. The basal medium was autoclaved and cooled to the room temperature under an atmosphere of N_2/CO_2 (90/10, *V/V*) and 30 mL/L bicarbonate solution (1 mol/L, autoclaved under CO_2), 1 mL/L vitamin, 1 mL/L trace elements mixture, and 1 mL/L selenite-tungstate solution were added (Widdel and Bak, 1992). In order to limit the growth of sulfate reducing bacteria, sulfate and sulfide were substituted by 100 μmol/L cysteine as a sulfur source.

Fresh soil sample (10 g) was put into 90 mL anoxic water and mixed homogeneously, then 5 mL of soil suspension was added to 45 mL mineral medium containing 30 mmol/L ferrihydrite and 10 mmol/L acetate under an atmosphere of N_2/CO_2 (90/10, *V/V*). Ferrihydrite was prepared using $FeCl_3$ and NaOH as described previously (Straub *et al*., 2005), and identified by X-ray diffraction (XRD). The cultures were incubated at 30°C in the dark. Standard anaerobic culturing techniques were used throughout the experiments (Widdel and Bak, 1992).

Iron reduction was indicated by the change in the medium color from orange brown to black due to the production of Fe(II), and Fe(II) was monitored overtime by the colorimetric reagent 1,10-phenanthroline (data not shown). In this study, the iron reducing bacteria was designated as HN.

1.3 Rice growth and iron plaque collection

Rice seeds (*Oryza sativa* L.) cv. Jiahua-1 were disinfected in a 30% H_2O_2 (*W/W*) solution for 10 min, and then washed by deionized water. The seeds were germinated in moist perlite. After three weeks, uniform seedlings were selected and transplanted into bags (mesh size × diameter ×height, 37 μm × 7.5 cm × 10 cm; n = 1 plant/bag) filled with 0.15 kg of quartz sand. The bags were placed in the center of 1.5 kg pots, and the gap between the nylon bag and the PVC pot was filled with 0.8 kg of soil. This allowed the separation of a rhizosphere/non-rhizosphere compartment from the soil compartment. After being transplanted into the PVC pot, the rice plants were grown in a greenhouse with a 10 h:14 h (light:dark) photoperiod. The temperature was kept at 25°C/20°C (day/night).

After 15 weeks, plants were harvested. Root samples were taken from rhizobags gently to avoid the damage of roots and iron plaque on the root surface. The root samples were washed with tap water to remove quartz sand and rinsed using deionized water more than three times.

1.4 Samples preparation

For the analysis of Fe and As concentrations in the iron plaque, some of fresh roots were extracted using dithionite-citrate-bicarbonate (DCB) as described by Liu *et al*. (2004b). Other parts of roots were freeze-dried for following experiments. The shade of red color of iron plaque accumulated on rice root surface was different from root tip to base. Root samples were cut into 1 cm segments, and mixed homogenously. For the analysis of total Fe and As in roots, some of these samples were digested with HNO_3 by a heating block (AIM600, A.I. Scientific Pty. Ltd., Australia). The remains were sterilized by autoclave to examine the microbial mediated iron plaque reduction and associated arsenic mobility. The Fe and As concentrations in iron plaque were 164.29 and 0.17 g/kg dry weight (dw), respectively, and in root were 276.25 and 0.20 g/kg dw, respectively.

1.5 Microbial iron plaque reduction

Iron plaque as the electron acceptor and acetate (final concentration 5 mmol/L) as the electron donor were added to the bicarbonate-buffered mineral medium with or without the addition of anthraquinone-2,6-disulfonic acid (AQDS, final concentration 100 μmol/L). AQDS, which acts as an electron shuttle to overcome the need for microbes to operate in direct contact with Fe(III)oxides, potentially can accelerate the reduction of insoluble minerals (Straub *et al*., 2005). In order to investigate microbial iron reduction, iron reducing bacteria were inoculated, and four treatments were conducted: (1) non-amended (control), (2) AQDS-amended (AQDS), (3) iron reducing bacteria-incubated (HN), (4) HN incubated and AQDS amended (HN + AQDS).

As positive control, arsenate (final concentration 150 μmol/L) and ferrihydrite (final concentration 7.5 mmol/L) were added to the mineral medium with or without the addition of AQDS (final concentration 100 μmol/L). Acetate (final concentration 5 mmol/L) was used as the electron donor for iron reducing bacteria. Hence, three treatments: non-amended (control), iron reducing bacteria-incubated (HN + As), and HN incubated and AQDS amended (HN + As + AQDS) were performed. Liu *et al*. (2006) demonstrated that As(V) predominated over As(III) in iron plaque, therefore arsenate was used in the experiment. A

10% inoculum was used throughout the two experiments, and cultures were incubated in the dark at 30°C. Each treatment was performed in triplicates.

1.6 SEM/EDS/XRD analysis of the iron minerals

The settled mineral residue was removed from the positive control described above and dried under anaerobic conditions. The dried solid was mounted onto a copper sample holder and coated with gold for the analysis of scanning electron microscopy (SEM, S-3000N, Hitachi, Japan). An energy-dispersive spectroscopy (EDS) system (EDAX Genesis, USA) was used to determine the major elements present in the biominerals. The mineralogy of the minerals was determined by X-ray diffraction (XRD). The dried solid was smeared on a glass slide and ground into fine slurry with the addition of a few drops of amyl acetate, and then the sample was analyzed using D/max2500 Diffractometer with CuK_α radiation (Rigaku, Japan). Slides were kept under anaerobic atmosphere until analysis.

1.7 Analytical techniques

The Fe and As concentrations in the iron plaque and root were measured by an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin Elmer, USA). Approximately 1 mL of sample was collected from growing cultures and acidified for 15 min with 0.5 mol/L HCl prior to iron analysis. Fe(II) in solution was monitored over time by the colorimetric reagent 1,10-phenanthroline. Total As in solution was measured by atomic fluorescence spectrometry (AFS-2202E, Beijing Haiguang Co., China) with 1 mL aliquots filtered with a 0.22-μm filter. Arsenic speciation was measured by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) (Zhu *et al*., 2008a). A reagent blank and standard reference plant and soil materials were included to verify the accuracy and precision of the digestion procedure and subsequent analysis using recovery. An internal standard was included and matrix effect was not observed for ICP-OES, atomic fluorescence spectrometry and HPLC-ICP-MS.

2 Results

2.1 Reduction of iron plaque by iron reducing bacteria

The medium containing acetate and the iron plaque was inoculated with iron reducing bacteria (HN), and the change of Fe(II) concentrations over time is shown in Fig. 1a. No significant difference between control and AQDS treatments was observed (data of Fe(II) concentration in the treatment with AQDS amendment were omitted). Iron plaque reduction commenced immediately following inoculation of HN, and Fe(II) concentration increased fast, especially in the treatment with AQDS. Although Fe(II) also released in the control treatment during the incubation, Fe(II) concentration of the treatment with AQDS and HN increased more faster than the control during the initial 7 d of incubation. During the terminal of the experiment (day 25–30), the Fe(II) concentration reached a relatively stable phase, and there was no significant difference between biotic treatments and control.

The release of aqueous As in the experiment with time is shown in Fig. 1b. Arsenic concentration in the control solution increased gradually in the first 20 d, and reached a relatively invariable phase between day 20 and day 30. Compared with control, aqueous As concentration in the HN treatment increased faster with first 10 d, then increased slower from day 11 to day 30. While As concentration increased sharply in the HN+AQDS treatment during the initial 8 d, then decreased gradually to about 13 μmol/L.

There was a significant correlation between Fe(II) and total As (Fig. 2). With increasing Fe(II) concentration As released quickly into the solution. The increasing rates calculated based on correlation equations was higher in control than that in biotic treatment.

The concentrations of As species in different treatments at the end of the experiment are shown in Table 1. The concentrations of As(III) and As(V) in the control were higher than those in the biotic treatments, and dimethyl arsenic (DMA) concentration in the control was lower than that in the biotic treatments. In addition, compared with

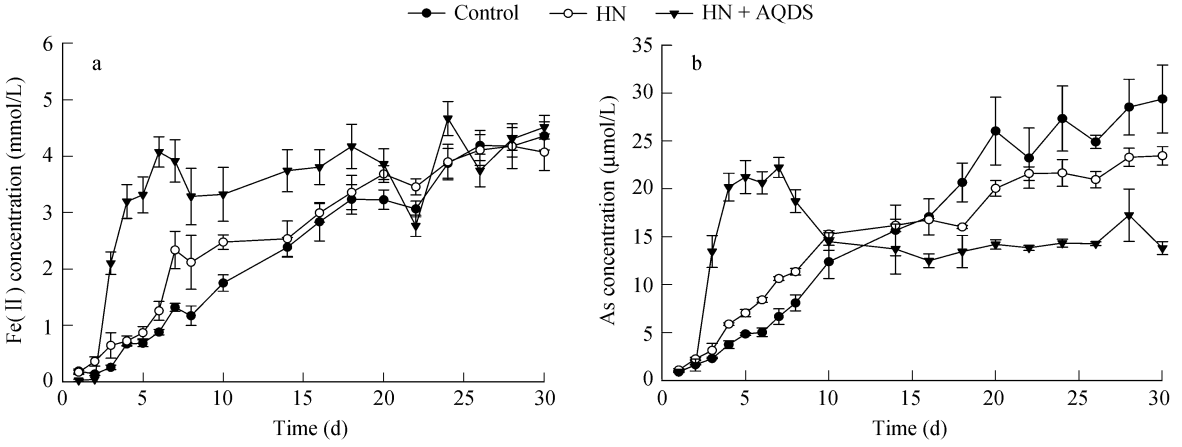

Fig. 1 Fe(II) (a) and total As (b) concentrations in the culture solution for different treatments with iron plaques as the electron acceptor over the incubation time. HN: inoculated with iron reducing bacteria, HN+AQDS: inoculated with iron reducing bacteria and AQDS; control: without bacteria. Error bar represent standard errors of three replicates.

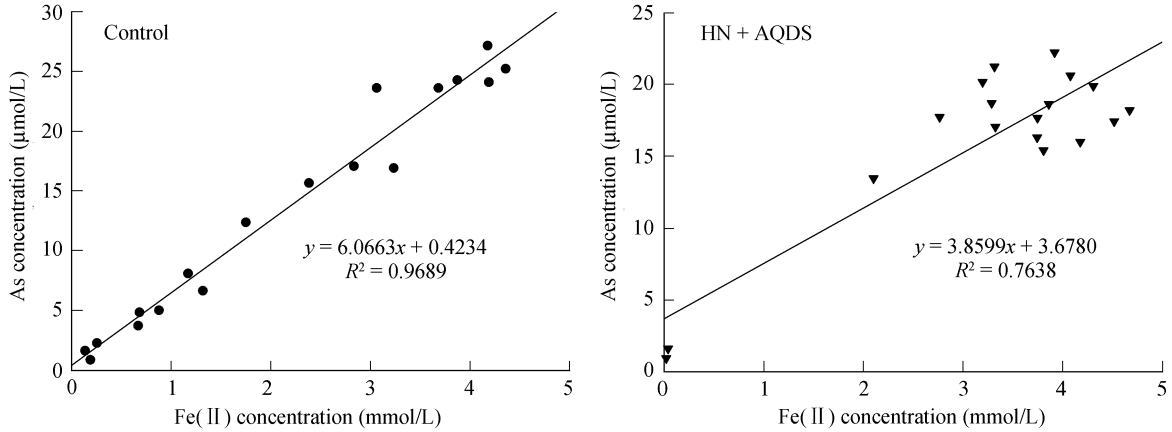

Fig. 2 Correlation between Fe(II) and total As in the culture solution for control and HN+AQDS treatment with iron plaques as the electron acceptor.

Table 1 Concentration of As species in different treatments at day 30

As species	Treatment		
	Control	HN	HN + AQDS
As(III) (μmol/L)	19.38	18.88	10.34
DMA (μmol/L)	0.86	0.98	1.79
As(V) (μmol/L)	3.36	3.12	2.87
Recovery (%)	93.6	98.0	86.1

DMA: dimethyl arsenic.
Recovery (%) = (the sum of As(III), As(V) and DMA)/As total × 100%.

the HN treatment, As(V) and As(III) concentrations were lower in HN + AQDS treatment, while DMA concentration was higher than that in HN treatment.

2.2 Interaction between arsenic and biogenic Fe(II)

The positive control experiment was conducted to investigate possible reasons of aqueous As retention during microbial iron plaque reduction process. Significant ferrihydrite reduction was observed over 8 d (Fig. 3a). The Fe(II) concentration began to increase at day 2 and reached the maximum concentration of 3.68 mmol/L at day 7 for the treatment with iron reducing bacteria. While in the biotic treatment supplied with AQDS, the maximum Fe(II) concentration was 4.88 mmol/L. Corresponding with Fe(III) reduction, the color of ferrihydrite in solution changed from red-brown to black, while no color change was observed in the control.

The total aqueous As concentration decreased sharply from initial 150 μmol/L to about 25 μmol/L after one day of equilibrium. Arsenic retention in the biotic treatment inoculated with HN was substantially higher than that in the control (Fig. 3b).

Iron minerals formed in the biotic system were collected from the solution, then imaged by SEM and analyzed by EDS. The results indicated that iron minerals were dominate, and high amount of As was absorbed (Fig. 4). The iron minerals were further analyzed by XRD. As shown in Fig. 5, new peaks were found in the minerals compared with ferrihydrite. Iron minerals were identified as γ-FeOOH. It demonstrated that amorphous minerals formed crystal minerals by iron reducing bacteria.

3 Discussion

This study described both chemical and microbial iron plaque reduction processes and their effects on As mobility. To our knowledge, the impact of microbial iron plaque reduction on As mobility is first reported in current study. During the iron reduction process, iron plaque

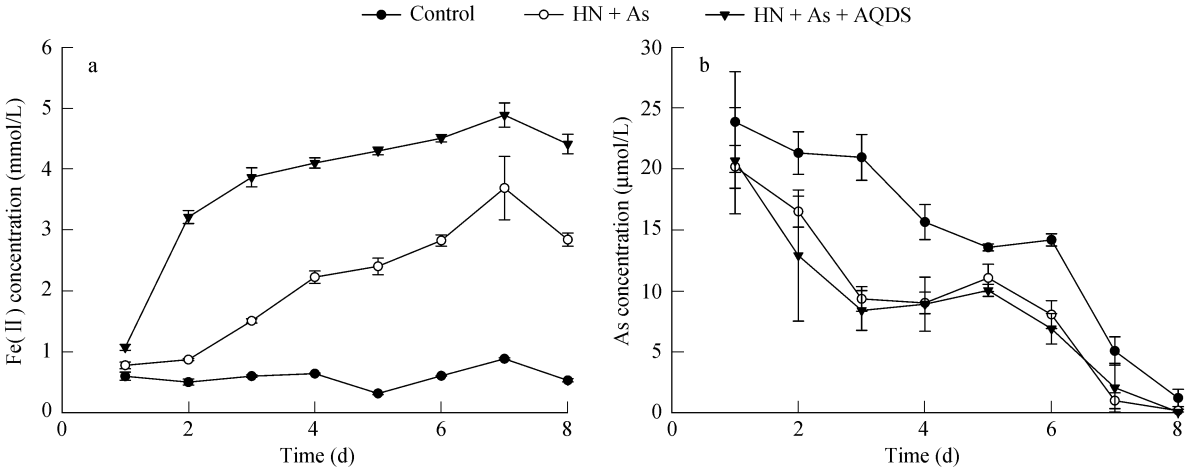

Fig. 3 Fe(II) (a) and total As (b) concentrations in the culture solution for different treatments with ferrihydrite as the electron acceptor over the incubation time. Error bar represent standard errors of three replicates.

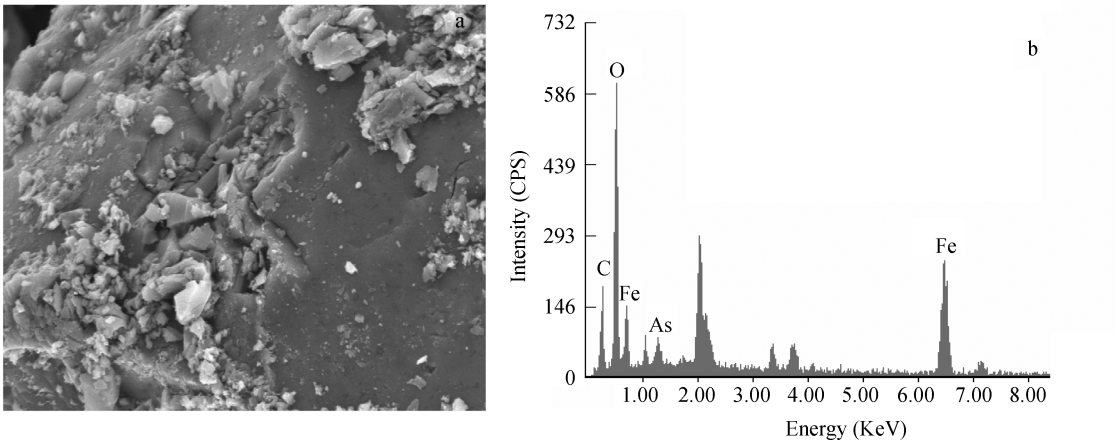

Fig. 4 Results of scanning electron microscopy (a) and energy-dispersive spectroscopy (b) of biogenic iron minerals collected from the medium supplement with ferrihydrite and iron reducing bacteria.

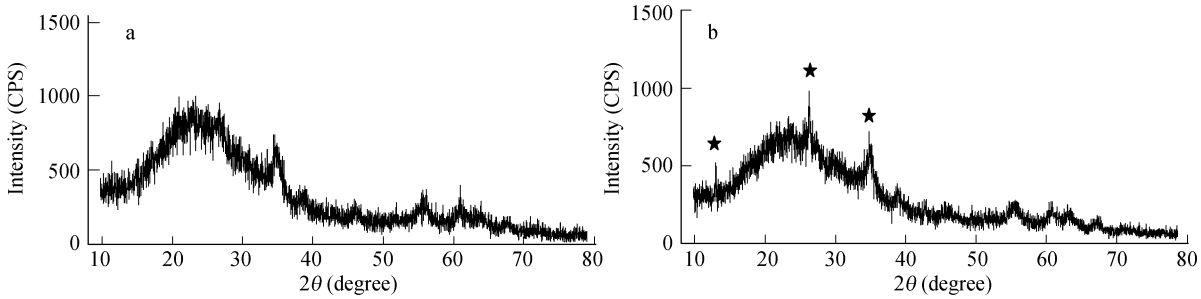

Fig. 5 X-ray diffraction investigation of ferrihydrite (a) and biogenic iron minerals (b). ★: peaks of crystalline iron minerals.

reduction also was observed in the control besides the biotic treatment. The reason is that root decay can release organic acid, and thus result in the dissolve and the reduction of Fe(III) minerals (Ratering and Schnell, 2000). In the present study, we observed a significant correlation between Fe(II) and total As in the solution. It confirmed that As release can be coupled with abiotic or biotic iron reduction, which was consistent with previous hypotheses that As mobilization was regulated by reducing and dissolving of Fe(III) minerals (Horneman *et al*., 2004; Islam *et al*., 2004; Chauhan *et al*., 2009). Surprisingly, although As released from iron plaque in the all treatments at the beginning, As retention was enhanced by iron reducing bacteria compared with abiotic control, especially in the HN+AQDS treatment (Fig. 1b). In addition, the concentrations of As(III) and As(V) in the abiotic treatments were higher than those in the biotic treatments at the terminal of the experiment (Table 1). These results demonstrated that iron reducing bacteria appeared to induce As sequestration within iron minerals by iron reduction. In order to explore the interactions between biogenic iron minerals and As, a positive control experiment was designed that As(V) was added to the medium with acetate as the electron donor and ferrihydrite as the electron acceptor for iron reducing bacteria. Microbial reduction of ferrihydrite induced the change in Fe mineralogy (Fig. 5), thus it had a large effect on arsenic retention owing to the reduction in surface area with the formation of more crystalline phase (Tufano and Fendorf, 2008; Shi *et al*., 2009). These results suggested that the reduction of Fe(III)oxides alone was not sufficient to mobilize As (Islam *et al*., 2005). Although a recent study suggested that such reductive process did lead to the As release into the aqueous phase over long time (Tufano and Fendorf, 2008), it was likely to control the incubation period and treatment systems to maintain effective As immobilization.

Several studies have observed that AQDS, a functional analogue for quinine moieties in humic acids, transfer electrons between microorganisms and iron minerals (Kappler *et al*., 2004; Jiang and Kappler, 2008). AQDS can be reduced by microorganism to AHQDS (anthrahydroquinone-2,6-disulfonic acid) which can act as a chemical reductant with concomitant regeneration of AQDS. Thus, it can enhance the reduction of Fe(III) and other metals (Fredrickson *et al*., 2000). In our study, AQDS accelerated Fe(II) production, and subsequently promoted As release from iron plaque in the biotic system at the initial period (0–7 d) (Fig. 1). Between day 8 and day 30, continued active iron reduction was companied by As decrease in solution compared with the abiotic treatments. These results suggested that by controlling the reaction period, on one hand, As can be extracted from As-bearing Fe(III)(hydr)oxides effectively, on the other hand, As release can be inhibited. Humic substances belong a chemically heterogeneous class of polymeric organic compounds that are widespread in upland and paddy soils (Dou *et al*., 2008) and iron oxyhydroxides are ubiquitous reactive constituents in subsurface environments, which are the preferred substrates for iron reducing bacteria (Straub *et al*., 2005). Therefore, these are possible ways to

remediate As contaminated soils and reduce potential risk for human by utilizing iron reducing bacteria.

Although As concentration in the soil used here was around 42 mg/kg, the concentration of As in iron plaque was much higher (up to 170 mg/kg, DCB method), demonstrating that the iron plaque formed naturally on the rice root surface could accumulate As. Liu *et al*. (2006) have demonstrated that As(V) predominated over As(III) in iron plaque, however, in current article, As(III) was predominant in the solution after microbial reduction of iron plaque (Table 1). The transformation of As species may be due to the microbial iron reduction or iron reducing bacteria, because some studies found that iron reducing bacteria also can respire As(V) (Islam *et al*., 2004; Kocar *et al*., 2006). Arsenic species during the whole reductive process should be monitored to illustrate mechanism of As transport during microbial iron plaque reduction process in further research.

4 Conclusions

This study investigated the microbial iron plaque reduction and its subsequent effect on As mobility. Microbial and chemical reduction of iron plaque caused As release from iron plaque to aqueous phase, however, microbial iron reduction induced the formation of more crystalline iron minerals, leading to a As sequestration. Additionally, the presence of AQDS in solution can accelerate the iron reduction, the As release from iron plaque and subsequently the As retention in the crystalline iron mineral.

Acknowledgments

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