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Distinct Function of Metal-Reducing Bacteria from Sediment and Groundwater in Controlling the Arsenic Mobilization in Sedimentary Aquifer

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Abstract

Microbially mediated dissolution of iron oxyhydroxides plays a defining role in the arsenic (As) release in most reducing environments, though the nature of this relationship remain unclear. This study aimed to evaluate the microbial processes on the enhancement or inhibition of As release as a function of bacterial enrichments from groundwater and core sediments. Two enrichment cultures of reducing bacteria (RB) used were cultivated from core sediment (RB-S) and groundwater (RB-W). The microcosm experiments were systematically conducted to assess microbially mediated reactions for the mobility of As from sediment into groundwater. From the analysis results, simultaneous bioreduction of As and Fe contributed to the initial elevation of aqueous As. Distinct distribution patterns of aqueous As and Fe between RW-S and RB-W revealed the different microbial activities. RB-W showed strong affinity for solid As, leading to high level of aqueous As. In contrast, RB-S exhibited high reducing ability toward Fe minerals, and the following formation of secondary Fe-As minerals constrained dissolved As. By amending with non-sterile groundwater, we also observed the chelating solubilization of As-contained Fe minerals, leading to the increase of As(III) and Fe(III). This comparative study illustrates the distinct function of indigenous metal-reducing microbes cultured from groundwater and sediment in liberating aquifer As and Fe. Our results provide evidence that the release and sequestration of As are closely related to specific microbial population in aquifer. The limitation of bioavailability of As and carbon source further influences the release of As to groundwater.

Keywords: Arsenic mobilization; Groundwater; Sediment; Microbial distribution; Reducing bacteria

Introduction

Elevated arsenic (As) concentration in soil and groundwater, particularly in South Asia, threatens the health of tens or hundreds millions of local residents relying on soil and groundwater as their primary food and water resources [1,2]. The host sediments are generally believed to be the immediate sources of arsenic in these areas [2]. Regarding the As release mechanisms, several hypotheses, including redox reactions and ionic competitions, have been proposed for controlling the As mobility [2-5]. Among them, the microbially mediated dissolution of As-bearing iron oxyhydroxides is the most plausible explanation for the occurrence of As in most anoxic environments [3-4,6-8]. The limited availability of organic carbon in groundwater and the finite amounts of As associated with reactive Fe oxyhydroxides are believed to be responsible for the heterogeneous distribution of As in groundwater [9,10].

A number of microcosm experiments using natural soil and synthetic minerals loaded with As [3,11-16] concluded that the microorganisms have a defining role in triggering As mobility. However, the mechanisms that influence dissolved As concentrations under anaerobic conditions vary considerably depending on the posited conditions [17]. Nonetheless, microbe-involved interplay with As-bearing Fe minerals leads to the principal elevation of pore-water arsenic concentrations, though the nature of these relationships remains contentious [18]. The dissimilatory microbial (enzymatic) reduction of Fe(III) minerals, predominantly through Fe(III)-reducing bacteria, is a terminal electron accepting process in numerous anaerobic environments [17,19]. The rate and extent of microbial reduction of Fe(III) oxyhydroxides is influenced by various factors, including the microbial community structure and biomass, the type and abundance of Fe(III) oxyhydroxides, and the sorption affinity between the oxide phases and bacteria [20].

Although microbial processes have been shown to liberate sedimentary As into groundwater, most studies focused on the contribution of microbial populations cultivated from sediment or soil. Planktonic or free-living populations, however, can transport with groundwater and disperse throughout the aquifer. Their roles in the mobilization of as remain obscure. Thus, the objective of this study is to assess the influence of reducing bacteria (RB) from the sediment and groundwater in controlling the mobilization of As in sedimentary aquifer. The distinct function of the indigenous microbes cultured from sediment and groundwater in retarding and enhancing the mobilization of sedimentary As is identified. Accordingly, an integrated conceptual model of biogeochemical cycling of As under reducing environment is postulated. The results of microcosm-based experiments can provide further understandings of microbial processes affecting the sequestration and mobilization of As in groundwater aquifers.

Materials and Methods

Site description and sampling

The southern Choushui river alluvial fan is located in the southwestern part of Taiwan (Figure 1a). Based on the subsurface

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hydrogeological analysis to a depth of around 300 m, the formation is divided into three marine sequences and three non-marine sequences in the distal-fan and the mid-fan areas [21]. The non-marine sequences of the formation, with coarse sediment, ranging from medium sand to highly permeable gravel, are typically regarded As aquifers, while the marine sequences of the formation with fine sediments, are considered As aquitards (Figure 1b). The aquifers are labeled by 1, 2 and 3 from top to bottom. The aquifer 2 is further divided into two sub-aquifers As 2-1 and 2-2 respectively due to the presence of a non-continuous aquitard. More detailed hydrogeological characteristics of this area have been described by Liu et al. [22]. Groundwater samples were collected from the monitoring well YL7 (23° 37' 48" N/120° 9' 19" E) located at the coastal area of the southern Choushui River alluvial fan [23]. The well screen was located in the uppermost aquitard at depths of 18-22 m (Figure 1c). The well YL7 was chosen because the previous surveys conducted by Tainan Hydraulics Laboratory, indicated that the groundwater in this well possessed the highest As concentration in this area (a mean of 450.4° g L^{-1} and ranges from 77.0 to 1469.1° g L^{-1}). Moreover, the groundwater in this well was characterized by the dissolved Fe level ranging from 0.1 to 5.1 mg L^{-1}, the SO_4^{2-} level from 1.0 to 316.0 mg L^{-1}, the nitrate level from 0.0 to 2.8 mg L^{-1} and the DOC level from 0.0 to 11.2 mg L^{-1} [24]. The detail chemical compositions of this well during 1992 – 2005 are shown in Table 1. Plots of major ions on the Piper diagram indicated that local groundwaters were characterized as NaCl or Na-Mg-HCO_3 type [8]. Groundwater was concurrently collected from the well YL7 while purging N_2 in order to minimize exposure to the atmosphere [25]. Sample pH values ranged from 7.0 to 7.6, and temperatures varied from 22 to 27°C. The sampled water was stored in sterilized and sealed serum bottles with no headspace left and used for subsequent enrichments of RB (RB-W). To cultivate sediment-related RB (RB-S) and conduct subsequent microcosm experiments, a new fresh core, YL7-1 (23° 34' 07" N/120° 10' 04" E), was drilled at a distance of 10 m from YL7 to a depth of 25 m without using drilling mud. A drill rig and a split-tube sampler with PVC liner (50 mm outside diameter) which had been pre-cleaned were used to collect sediment core. Cores were extruded and segmented by depth in an anoxic, sterilized glove

Parameters	Minimum	Maximum	Average	Standard deviation
pH	7.5	8.4	7.9	0.2
EC(° s/cm)	0.7	4010.0	2209.0	912.2
TDS(mg/L)	430.0	2955.0	1432.4	626.2
DO(mg/L)	0.0	6.8	1.61	1.36
DOC(mg/L)	0.0	11.2	4.3	2.2
Ca^{2+}(mg/L)	28.0	123.0	59.3	19.8
Mg^{2+}(mg/L)	15.0	119.0	73.2	30.3
Na^{+}(mg/L)	107.0	460.0	293.5	90.8
K^{+}(mg/L)	7.0	73.0	38.7	15.5
Total As(° g/L)	77.0	1469.1	450.4	314.3
Fe(mg/L)	0.1	5.1	0.8	1.1
NH_4^{+}(mg/L)	0.0	16.8	7.1	4.1
NO_3^{-}(mg/L)	0.0	2.8	0.5	0.6
NO_2^{-}(mg/L)	0.0	13.5	1.4	2.9
HCO_3^{-} (mg/L)	191.0	650.0	504.4	84.8
Cl^{-}(mg/L)	19.0	802.0	391.9	215.6
SO_4^{2-}(mg/L)	1.0	316.0	102.9	82.8

Table 1: Summary of aquitard groundwater quality in monitoring well YL7 during 1992-2005.

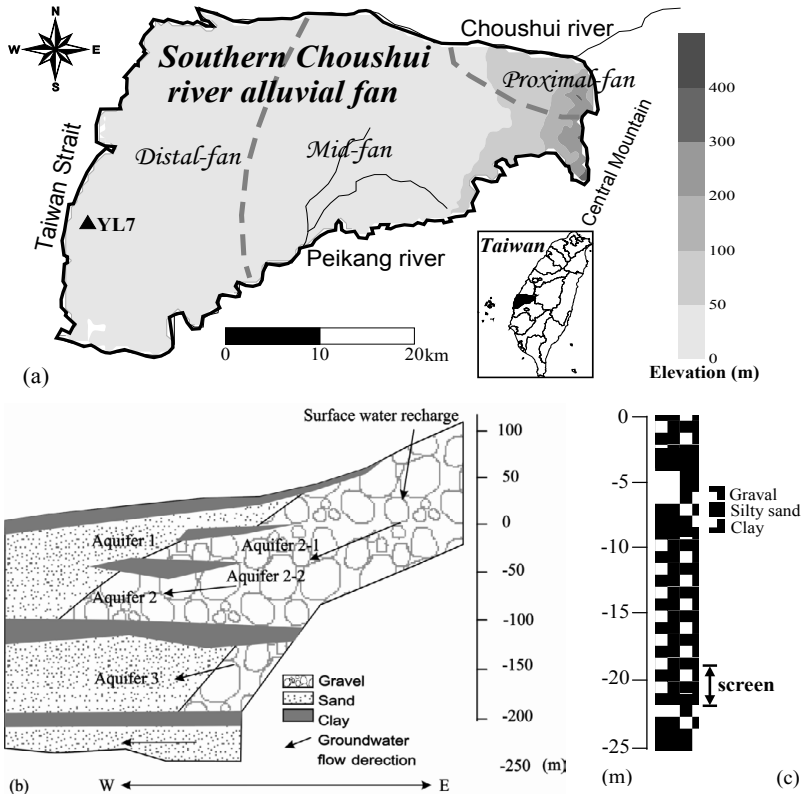

Figure 1: Sampling location of monitoring well YL7(▲). (a) Geographical elevations, (b) hydrogeological profile of the southern Choushui river alluvial fan, and (c) location of well screen and lithology of YL7.

box containing N_2 gas. Core samples were dark in color, and consisted of silty sand, mud, and clay. The C-14 dating data show that the sequence formation is in the Holocene age [22]. Only the sediments retrieved from depths of 19.5-20.0 m were used for enrichment. Solid As ranged from 4.02 to 12.77 mg kg^{-1}, with a mean of 7.21 mg kg^{-1}; whereas the Fe contents varied from 2.5 to 4.6%, with a mean of 3.5%. The amounts of organic and inorganic carbon in the collected core sediments were comparable and generally less than 0.5% [4]. Groundwater and core sediment samples were stored at 4°C in polyethylene bags to minimize microbial activity. Further groundwater and sediment manipulation were performed only under strict anoxic condition.

Bacterial strains genus identification

Before conducting the microcosm experiments, identification of microbial population in the groundwater (YL7) and core sediment (YL7-1 at depth of 20 m) were firstly conducted. To enrich bacterial populations, groundwater sample of YL7 was inoculated into a chemically defined medium (CDM) (8.12 mM $MgSO_4$, 18.7 mM NH_4Cl, 7 mM Na_2SO_4, 0.0574 mM K_2HPO_4, 0.457 mM $CaCl_2$, 44.6 mM Na-lactate, 0.012 mM $FeSO_4$, and 9.5 mM $NaHCO_3$, with the pH adjusted to 7.2) [26] amended with or without 2 mM of As(III) or 10 mM of As(V) under aerobic and anaerobic incubation, respectively. Samples were then incubated at 25°C for 24 h. A pure culture bacterial genomic DNA was prepared as described by Wilson [27] with slight modification [28]. Briefly, bacterial pellets were collected from 1.5 ml of bacterial cultures, and resuspended in 564°L of tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.0), 30° L of 10% sodium dodecyl-sulfate (SDS), and 6° L of 10 mg/ml proteinase K, and incubated at 37°C for 1h. The solution was mixed with 100° L of 5M NaCl, and then mixed thoroughly with 80° L of a NaCl/cetyltrimethyl ammonium bromide (CTAB) solution. The solution was incubated at 65°C for 10 min. Genomic DNA was extracted with chloroform/isoamyl alcohol (1:1) and phenol/chloroform/isoamyl alcohol (25:24:1). DNA was precipitated with an equal volume of isopropanol, washed with 75% ethanol, and resuspended in 50° L of distilled H_2O.

The PCR amplification of 16S rRNA was performed using bacterial genomic DNA as a template. Universal primers were used to amplify 16S rRNA. The amplified PCR products were purified using a BioMan kit (GeneTeks, Taipei, Taiwan). The presence of 16S rRNA genes in the PCR product was confirmed by sequencing with ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Gene sequences were analyzed against the NCBI database using the BLAST program and matched to known 16S rRNA gene sequences. In addition to BLAST searches, the bacterial 16S rRNA gene sequences were analyzed using the RDP (Ribosomal Database Project) classifier (http://rdp.cme.msu.edu). The bacterial 16S rRNA clones were then assigned to a genus, a family or an order based on the confidence of the combined resulted obtained.

For sediment cultures, about 15 g of YL7-1 sediment (from 20 m in depth, dark gray fine sandy loam) was mixed with 30 mL of artificial groundwater (1.97 mM $MgCl_2$, 0.33 mM $MgSO_4$, 0.51 mM $NaHCO_3$, 0.01 mM $NaNO_3$, 0.01 mM K_2HPO_4, and 5.50 mM $CaCO_3$, with the pH adjusted to 7.0 with concentrated HCl) which constituents are similar to groundwater samples near the study site. After 8 weeks of incubation, sediments DNA amended with and without acetate were extracted and analyzed for microbial communities [29]. Sediment DNA was extracted from 1 g of sediment using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The 16S rRNA fragment (645 bp) was amplified by a polymerase chain reaction (PCR) from extracted DNA samples using universal bacterial primers [30]. PCR amplifications were performed in a 50° L reaction volume containing 0.2 ° M of each primer, 1.5 mM of Mg^{2+}, 0.2 mM of dNTPs, and 2 U of Taq DNA polymerase (GeneTeks, Taipei, Taiwan). The purity of the amplified product was determined by gel electrophoresis and the amplified PCR product were purified using a BioMan kit (GeneTeks, Taipei, Taiwan). The purified PCR product yT&A vector using a TA cloning kit and competent Escherichia coli cells (Yeastern Biotech, Taipei, Taiwan) according to the manufacturer's instructions. Gene sequences analysis methods were the same as used for the groundwater samples analysis. The identified bacterial strains in the cultured groundwater and sediment samples were listed in Table 2. Detailed description of the bacteria strains genus identification can refer to Liao et al. [28,29].

Enrichments of RB

Two RB enrichment cultures were performed using core sediment (YL7-1) and groundwater (YL7) as inocula. The medium was designed using acetate and lactate (10 mM) as the electron donors, and Fe(III)-citrate (20 mM) as the sole electron acceptor. Other compositions in the designed medium included basal salts, vitamins, and trace elements (Table 3). The enrichment cultures of RB were subculturing in fresh medium with a minimum of four times in order to reduce the potential interference associated with inoculated sediments or groundwater. These enrichments are denoted as RB-W and RB-S based on their inoculation with groundwater and core sediment, respectively.

Microcosm experiments for As release

The microcosm experiments were designed as a function of microbial population and sediments. All the tests were prepared as follows: 10 g of sediment was added to sterile 160-mL serum bottles containing 80 mL of water solution under anoxic conditions. All bottles were acid-washing and sterilized before use. Two sets of microcosm

16S rRNA genus identification	Origin		
	groundwater	sediment unamended	sediment amended with acetate
Pseudomonas sp.	+[1]	+	+
Psychrobacter sp.	+	-[2]	-
Vibrio sp.	+	-	-
Citrobacter sp.	+	-	-
Enterobacter sp.	+	-	-
Bacillus sp.	+	-	-
Bosea sp.	+	-	-
Caulobacter sp.	-	+	+
Rhizobium sp.	-	+	-
Rhodobium sp.	-	-	+
Brachymonas sp.	-	+	-
Escherichia sp.	-	-	+
Natronocella sp.	-	-	+
Shewanella sp.	-	+	+
Desulfuromonas sp.	-	+	-
Alkalibacterium sp.	-	+	+
Clostridium sp.	-	+	+
Gracilibacter sp.	-	+	-
Iron-reducing bacterium enrichment culture clone HN-HFO4	-	-	+

[1]Found in the analyses.
[2]Not found.
Table 2: Bacterial strains genus identification of groundwater (YL7) and core sediment (YL7-1) samples.

Basal salt (g L^{-1})	
NaCl	1.17
$MgCl_2 \cdot 6H_2O$	0.40
KCl	0.30
$CaCl_2 \cdot 2H_2O$	0.15
NH_4Cl	0.27
KH_2PO_4	0.20
Electron acceptor and carbon sources (mmol L^{-1})	
Ferric citrate	20
Lactate	10
Acetate	10
pH buffer (mmol L^{-1})	
Bicarbonate	25
Vitamin solution (g L^{-1})	
Biotin	2.0×10^{-4}
Folic acid	2.0×10^{-4}
Pyridoxine-HCl	10^{-3}
Thiamine-HCl·$2H_2O$	5.0×10^{-4}
Riboflavin	5.0×10^{-4}
Nicotinic acid	5.0×10^{-4}
D-Ca-pantothenate	5.0×10^{-4}
Vitamin B12	10^{-5}
p-Aminobenzoic acid	5.0×10^{-4}
Lipoic acid	5.0×10^{-4}
Trace metals (g L^{-1})	
Nitrilotriacetic acid	1.5×10^{-4}
$MnCl_2 \cdot 4\ H_2O$	5.2×10^{-3}
$CoSO_4 \cdot 7\ H_2O$	1.8×10^{-3}
$NiCl_2 \cdot 6\ H_2O$	2.0×10^{-4}
$CuSO_4 \cdot 5\ H_2O$	10^{-4}
$ZnSO_4 \cdot 7\ H_2O$	2.0×10^{-3}
$Na_2MoO_4 \cdot 2\ H_2O$	3.0×10^{-4}
Na_2SeO_4	3.0×10^{-4}
$AlK(SO_4)_2 \cdot 12\ H_2O$	3.0×10^{-4}
H_3BO_3	10^{-4}
Redox indicator (1 mL : 1 L)	
Resozurin	1

Table 3: Compositions of media used for RB enrichment.

experiments were performed. One set was designed to evaluate the reductive ability between medium-related RB and amending sediments with artificial groundwater (1.17 g L^{-1} NaCl; 0.40 g L^{-1} MgCl ·6H O; 0.30 g L^{-1} KCl; 0.15 g L^{-1} CaCl ·2H O; 0.27 g L^{-1} NH Cl; 0.20 g L^{-1} KH PO). RB enrichment cells were washed three times with sterile, O_2-free DI water. After the final wash, the cells were re-suspended in a basal salt medium for inoculation and 1 mL solution was injected into the bottles under anaerobic condition. An equivalent volume of washed cells was inoculated for all biotic sets. Two cores from well YL7-1 at different depths, 16- and 18-m, which have high As contents (9.0 and 12.1 mg kg^{-1}) were used. For the controls, the cell suspension inoculation was replaced with the equivalent volume of sterilized artificial groundwater. Before the experiment, the sediments were suspended with media at least two days.

For the set using the in-situ groundwater as dominant solution, only one depth of the core (18 m) was utilized. Under biotic condition, the sediment was resuspended in the non-sterilized groundwater, with or without sodium acetate as reactive carbon source. The experiment results of these sets were also compared to those incubated with RB-W and sterilized groundwater. For abiotic condition, the sediment amended with the sterilized groundwater was treated as control. Competitive effect of sodium acetate was also examined.

All bottles were not stirred during incubation, but they were shaken vigorously before each sampling. All experiments were performed in duplicate.

Sampling and analyses

To ensure anaerobic conditions, all samples for Fe and As assays were collected periodically in the anaerobic chamber. Collected fluid samples were filtered using 0.22° m nylon filters. To minimize the potential oxidation, an aliquot of the sample was acidified with 1 N HCl at a ratio of 1:1. Total Fe was measured using inductively coupled plasma and atomic emission spectrometry (ICP-AES) (Varian VISTA-MPX). Whereas ferrous (Fe^{2+}) concentrations were measured colorimetrically using the ferrozine method [31]. Aqueous Fe (III) concentration was calculated by taking the difference between total Fe and Fe (II). Two major As species, As(III) and As(V) were separated using an anion column (Phenomenex, Nucleosil, 10 ° m, 250 × 4.6 mm), connected with a high performance liquid chromatography (HPLC) (Perkin-Elmer, Series 200), which was interfaced to an electro-thermal atomic absorption spectrometer (AAS) (Perkin-Elmer, AA 200) and a hydride generation (HG) system (Perkin-Elmer, FIAS 100) [32]. The recovery rates of As(III) and As(V) were 100.7 ± 3.8% and 97.2 ± 4.0%, respectively. The limited sample volumes available preclude the determination of other key analytes in the microcosms.

The contents of As and Fe in the cores were extracted from 1 g of sediment with 0.2 M NH_4^- oxalate buffer (pH 3.0) as previous described [33]. The arsenic concentration was analyzed using an AAS-HG system. Subsequently, 0.5% $NaBH_4$ in 0.25% NaOH and 1 M HCl were added to reduce arsenic to arsine. A flame atomic absorption spectrometer (Perkin-Elmer AA 100) was then employed to analyze the total Fe concentration.

X-ray absorption near-edge structure (XANES) spectra was used to determine arsenic speciation in the sediment after the incubation experiment. X-ray absorption spectra (XAS) at the As K-edge (11,867 eV) were collected at the Wiggler 20 beamline BL-17C at the National Synchrotron Radiation Research Center (NSRRC), Hsin-Chu, Taiwan. The Wiggler 20 beamline (17C), with an energy range of 4 to 15 keV, employed a Si(111) double-crystal monochromator for energy scanning using a resolving power (E/ΔE) of 7,000 and beam intensity of approximately 10^9~10^{10} photons per second. All samples were fixed onto an aluminum holder, sealed with Kapton tape, and placed at 45° to the X-ray beam. Sample spectra regarding the As K_α edge of 11,867 eV were collected from -50 to +100 eV, and then compared to XANES spectra of selected reference standards, including arsenate ($Na_2HAsO_4 \cdot 7H_2O$) and arsenite ($NaAsO_2$). In addition, the Au L_3-edge spectrum was monitored by the I_r chamber with I_t and I_f chambers simultaneously. The I_r chamber served as the reference for calibrating energy shifts because of monochromator drifts. Eight scans of each sample were processed and averaged to improve the data quality of XAS spectra. The Athena program was employed for standard background subtraction and edge-height normalization using the AUTOBK algorithm [34].

Results and Discussion

Characterization of the microbial communities in groundwater and sediment

Bacterial strains identification using 16S rRNA genus techniques

of groundwater (YL7), unamended core sediment (YL7-1) and core sediment (YL7-1) amended with acetate are reported in Table 2. Seven genera including *Pseudomonas sp., Psychrobacter sp., Vibrio sp., Citrobacter sp., Enterobacter sp., Bacillus sp. and Bosea sp*. were found in groundwater. Bacterial strains identified in unamended sediment and sediment amended with acetate were similar, but exhibited contrasting bacterial community between groundwater and sediment medium (Table 2). Only the Pseudomonas sp. appeared in both groundwater and sediment medium. *Pseudomonas sp*. species is a facultative anaerobe able to respire using alternative electron acceptors including nitrate but not Fe(III) or As(V). Other genera identified in the sediment with and without acetate amended were *Cavlobacter sp., Shewanella sp., Alhalbacterium sp. and Clostridium sp.*.

Microbial behavior and interactions

The reducing ability of three microbial populations, including RB-W, RB-S, and the bacterial population pertaining to YL7 groundwater, were evaluated. From the analytical data, the levels of As and Fe species concentrations were relevant to not only the source of RB enrichments but also the used core sediments (Figure 2). For the sets using 16-m core sediment, the most significant increase of As concentrations occurred within the first month of the incubation with RB enrichment cultures (Figure 2a,b). During inoculation with RB-W, the initial effluent of arsenic species, principally As(III), was concomitant with dissolved Fe(II), implying the congruent reduction of As and Fe (Figure 2a). Concentrations of As(III) and Fe(II) reached systematic equilibrium after the sixth sampling (approximately 42 days), with 60° g L^{-1} of As(III) and 28 mg L^{-1} of Fe(II). By contrast, this study observed distinct peak values of As and Fe species in the set inoculated with RB-S (Figure 2b). In this set, the maximum release of As(III) (ca. 20° g L^{-1}) occurred at the third sampling, where the remarkable increase of Fe(II) (up to 80 mg L^{-1}) was measured (Figure 2b). After this sampling, As(III) level gradually decreased to less than 10° g L^{-1} and the experiment reached equilibrium. Meanwhile, this set observed a simultaneous decrease in Fe(II), which stabilized at 60 mg L^{-1}. A kinetic formation of secondary Fe minerals accompanying the adsorption and/or coprecipitation of As can explain the dramatic decline in soluble Fe and As [15,16]. Negligible arsenic and iron mobilization was observed in the uninoculated sterile control (Figure 2c).

A large difference of As released between sets amending 16-m and 18-m core sediment was observed. Inoculation with RB-W resulted in the parallel increase of As and Fe after the second sampling (Figure 2d). As and Fe were subsequently equilibrated at the sixth sampling with 180° g L^{-1} of As(III) and 28 mg L^{-1} of Fe(II). Regarding the RB-W treatments, the distinct difference in peaky As(III) concentrations combined with similar amount of dissolved Fe(II) unraveled the influence of heterogeneous distribution of As content onto the surface of Fe oxyhydroxides within the used sediment (Figures 2a,d) [10,16]. For experiments with RB-S, a notable increase of Fe concentration resulted in a moderate level of As, compared to that observed in the microcosm with 16-m core sediment (Figure 2b,e). The peak value of As(III) (55° g L^{-1}) was measured at the third sampling, followed by the temporary stable state, which gradually decreased to 20° g L^{-1} at the end of incubation (Figure 2e). A similar tendency was also observed in the Fe(II) concentration, which reached a maximum value of 75 mg L^{-1} at the third sampling, and stabilized at a value of 55 mg L^{-1} after the experiment. No obvious elevation of the As or Fe species was measured in the control set (Figure 2f).

The indigenous microbial activities, particularly for groundwater population, exhibited strong reducing ability for arsenic in this area [28-29]. However, the role of organic carbon in As-contaminated aquifer remained under debate [35]. Further microcosm experiments were designed to assess the interplay between in-situ groundwater and labile carbon source. For the incubation with non-sterilized groundwater, initial level of As (III) was converted from aqueous As(V) (Figure 3a). Parallel increase of As and Fe started at 14 days, reaching up to 320° g L^{-1} for As(III) and 15 mg L^{-1} for Fe(III) at 35 days. Subsequently, nearly half amounts of As (III) were re-adsorbed, whereas the Fe(III) concentration remained stable. This result implied the re-adsorption capability of As under reducing environment [36]. Using acetate as a potential electron donor for metal reduction and a proxy for organic carbon influenced the trends of As and Fe remarkably (Figure 3b). Initially, rising levels of As(III) (up to 180° g L^{-1}) accompanied the congruent increase of aqueous Fe(III) (up to 15 mg L^{-1}). After one month incubation, gradual decline of Fe(III) concentration accompanying increase of aqueous As(III) suggested the decouple reactions of As and Fe [37]. Decrease of dissolved Fe accompanying the observation of color change to black on the surface of the core implied the formation of secondary Fe minerals such as magnetite [38]. Moreover, the addition of organic carbon improved the release of as significantly as described by the previous

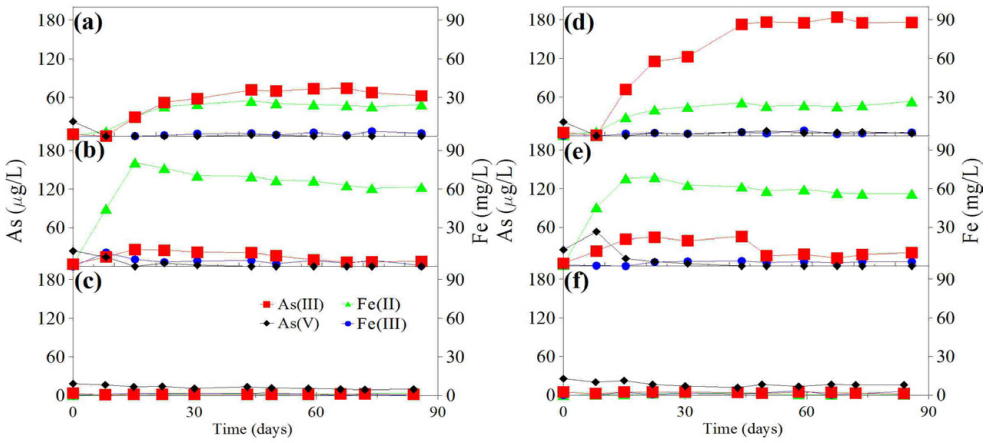

Figure 2: Variations of As(III), As(V), Fe(II), and Fe(III) concentrations in microcosm experiments incubated under a range of biogeochemical conditions. (a) 16-m sediment with RB-W; (b) 16-m sediment with RB-S; (c) abiotic control of 16-m sediment; (d) 18-m sediment with RB-W; (e) 18-m sediment with RB-S; and (f) abiotic control of 18-m sediment.

report [35]. Liao et al. [29] conducted the microcosm experiment using the indigenous sediments amended with or without acetate. According to the results, the congruent reduction of Fe and As led to the major release of As. Further, addition of organic carbon limited the As mobilization. Note that the combined results of this study and Liao et al. [29] indicated the distinct microbial activities occurred between groundwater and sediment, which were also proved by the analysis of bacterial microorganisms (Table 2). Adding acetate altered the terminal electron acceptors as observed herein indicating the shift of microbial community as reported by Islam et al. [3].

In assays of RB-W mixed with sterile groundwater, the trends of As and Fe species were comparable to that inoculated with the same population but amending with artificial groundwater (Figures 2b,2e and 3c). The similar levels of dissolved As in the abiotic sets, with and without acetate, revealed that competitive desorption by organic carbon, if it occurred, was insignificant under the experimental condition (Figures 3d,3e).

Overall, only biotic sets with and without addition of acetate showed noticeable increase in dissolved As and Fe; this finding supports the role of microorganisms in mediating the cycle of As and Fe under reducing conditions [3,11,39] (Figures 2 and 3). The discrepancy between the RB-W and RB-S experimental results is due to the contrasting bacterial communities cultured separately from groundwater and core sediment (Table 2) [28-29]. Different microorganism can utilize sedimentary As via various reaction pathways and influence the groundwater As concentration. In this study, we observed that RB-W population directly mediated the coupled reducing reaction of As and Fe in the sediment leading to a systematic increase of As (III) and Fe (II) concentrations. On the other hand, RB-S population exhibited strong reducing ability toward Fe minerals, resulting in dramatic increase of Fe(II) and formation of secondary As-bearing iron oxyhydroxides. It is noteworthy that the decouple reduction of As and Fe via the chelating solubilization of Fe minerals observed in this study provided another potential mechanism for the mobility of sedimentary As.

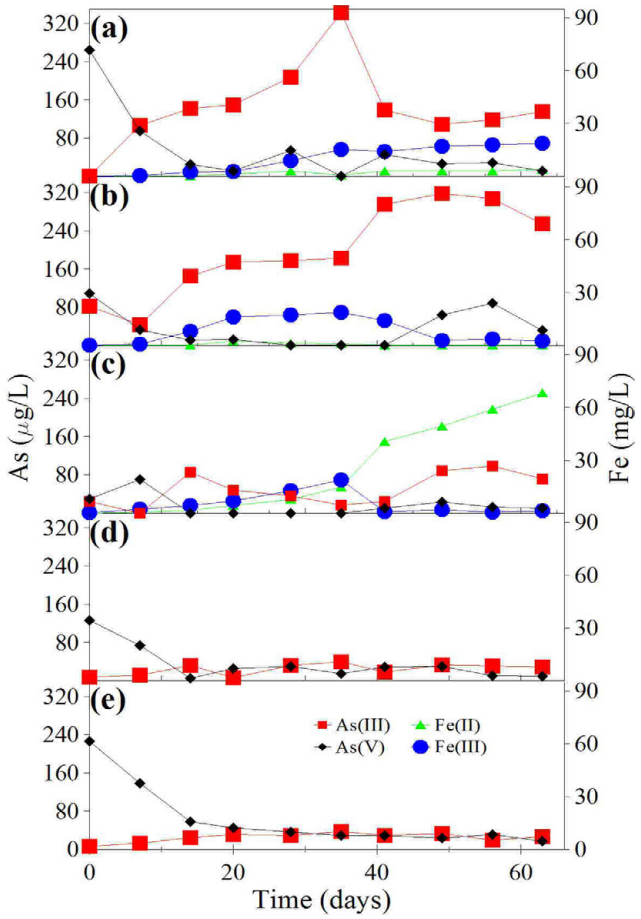

Figure 3: Variations of As(III), As(V), Fe(II) and Fe(III) concentrations in microcosm incubation experiments containing sterilized core sample (18 m) amended with (a) non-sterilized groundwater, (b) non-sterilized groundwater and acetate, (c) sterilized groundwater and RB-W, (d) sterilized groundwater and acetate and (e) sterilized groundwater only.

By combining the data of microcosm experiments with RB enrichment cultures, the discrepancy results of As and Fe between the experimental sets were attributed to the contrasting bacterial community between groundwater and sediment. (Figure 2 and Table 2). Reducing bacteria from groundwater prefer to release both Fe(II) and As(III) to aqueous phase whereas reducing bacteria from sediment prefer to only reduce Fe(II) and sequestrate As to solid phase. Further, the dominant dissolved Fe as Fe(II) or Fe(III) in this study revealed various reactive pathways toward Fe minerals [17,19,40-42]. Nonetheless, either through chelating solubilization or reducing dissolution of Fe minerals was regarded as one kind of reductive pathways [17].

Microbial effects on the As and Fe cycling

Three possible arsenic mobilization mechanisms mediated by metal-respiring bacteria have been proposed [39]. These microbial reactions are driven by degraded organic carbon using As(V), Fe(III), or both as terminal electron acceptors. For the RB-W inoculated treatments, microbial reduction of sedimentary Fe and As seemed to prevail under the biotic conditions (Figures 2a,2d). In the YL7 groundwater, a total of 7 arsenic-transforming bacterial strains have isolated (Table 2). Furthermore, *Psychrobacter sp.*, an identified arsenic-reducing strain herein, was also capable of reducing iron. According to the XANES results, the pristine valence of arsenic in the sediments was As(V) (Figure 4). After the experiment with RB enrichments, excluding the set amended with 16-m core sediment and incubated with RB-W, the predominant valence of solid arsenic switched from As(V) to As(III) with reference to that of control sets (Figure 4). These results reveal that the reduction of sedimentary As(V) is concomitant with the formation of secondary phase As(III)-bearing Fe minerals [39,42]. Accordingly, the observed coexistence of a pristine As(V) solid phase assemblage with an As(III)-dominated aqueous phase, combined with the moderate increase of dissolved Fe(II), evidences the coupled reducing reactions of As and Fe in sediment inoculated with RB-W populations (Figures 4 and 2d) [3,39].

Based on the sequence analysis by Liao et al. [29], the major clones in sediment of YL7-1 were affiliated with known members of α- and γ-proteobacteria and fimicutes. From the report, they concluded that sedimentary microorganisms mediated the release of As into groundwater and the addition of acetate influenced the microbial reducing processes. High level of As(III) with moderate Fe(II) concentration was observed without labile carbon source. By adding acetate as a potential electron donor for metal reduction and a proxy for organic matter caused a further increase in aqueous Fe (II) but dissolved arsenic decreased. Interestingly, while inoculating with RB-S, our study showed the same result, implying that the existence of Fe-reducing population, such as Shewanella sp. and iron-reducing bacterium enrichment culture clone HN-HFO4, as identified herein were responsible for the increase of Fe(II) (Table 2). Although the

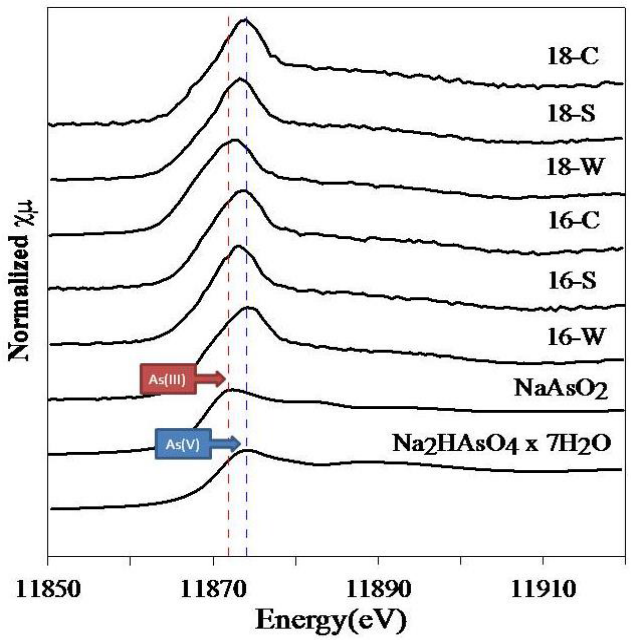

Figure 4: Normalized As XANES spectra of the sediments after the experiments with IRB populations. The number represents the depth of sediment used, the capital letters W and S represent the inoculation of RB-W and RB-S, and C denotes the controls.

amount of dissolved Fe(II) measured in sets with RB-S was double that of RB-W treatments, only minor levels of As(III) were detected regardless of the sediments type (Figure 2). Based on these results, the metabolic pathway of sediment microbial community showed strong affinity for Fe, and the potential formation of secondary Fe minerals seemed to sequestrate As [43]. Thus, we concluded that the exertion of bacterial communities of these two RB enrichments expresses different metabolic pathways toward terminal electron acceptors on the incubation experiments. In short, the reducing bacteria cultured from groundwater prefer to mediate and release of both As (III) and Fe (II) whereas the reducing bacteria from sediment prefer to only reduce Fe (III) to F (II) and form As-bearing iron oxyhydroxides minerals.

For the sets suspended with in-situ groundwater, an unusual release mechanism of As that converted sedimentary arsenic and iron into solubilized As(III) and Fe(III) were observed (Figures 3a and 3b). Most studies focused on the reducing dissolution of As-bearing Fe oxyhydroxides in As-contaminated area but overlooked the other possible release pathway such as the chelating solubilization of Fe minerals [44]. Moreover, the mobility of As and Fe was decoupled processes, especially with the aid of reactive carbon source. Decoupling release of As and Fe in aqueous phase generally reflected adaption of the respiratory pathways in the sediments, through dynamic changes in species which were metabolically active in the microbial community, or through altered expression of the relevant metal reductases in key anaerobes constituting this community [3]. On the thermodynamic basis, the general sequence of microbial reduction of as (V) is followed by Fe(III) or sulfate [45]. We thus propose that the supplement of organic carbon acts as a key role in regulating the decoupled process, which results in a significant level of aqueous as with minor dissolved Fe in groundwater (Figures 3a and 3b).

Implications for *in-situ* environments

Microbial effects on liberating as from sediments were investigated under a series of microcosm experiments. The results of microcosm experiments using RB-W and RB-S enrichment cultures exhibit different reducing behaviors toward solid Fe and as. According to Figures 2a,2b the microcosm experiment of RB-W increased the aqueous As and Fe concentrations from 0 to 65° g L^{-1} and 0 to 24 mg L^{-1} , whereas the RB-S increased the As and Fe concentrations form 0 to 11° g L^{-1} and 0 to 61 mg L^{-1}, respectively. The RB-W enhances the liberation of Fe and as from sediment and the RB-S liberates the Fe but sequestrates the mobilization of As from sediment. The distinct function of microbes cultured from groundwater and sediment markedly affect the movement of as in a sedimentary aquifer.

The availability of labile organic carbon as a driving force for microbial reduction is possibly the most prominent in realizing the mobilization and distribution of As in groundwater [35,46]. Under anaerobic conditions, reducing bacteria used a variety of organic carbon As the carbon source and electron donor in gaining energy from redox couples. According to the previous study, the addition of organic carbon source resulted in the shift of original microbial population in sediment and limited the release of sedimentary as [29]. In this study, the influence linked to the supplement of labile organic carbon has also been recorded (Figures 3a,3b). The redox processes motivated by microorganisms responded dynamically to the addition of organic carbon, and subsequently contributed to the variation of measured components (Figures 3a,3b). This decoupled process was consistent with our previous study [4] and implicated the potential contribution of microbial community in groundwater on triggering the release of As in the subsurface. The experimental results offered another explanation for the poor relationship between As and Fe in aqueous phase in this area. Further, the competitive effect of organic carbon against the As onto the surface of Fe minerals in this area was eliminated (Figure 3d). Difference in the Fe reduction and as(III) release profiles between our previous study [29] and this study with the addition of acetate could be explained in terms of the contrasting microbial community (Table 2) and their interactions with the distinct geochemical environments in the microcosm experiments.

This study showed the influence of heterogeneous distribution of As-bearing Fe minerals, which could also serve as potential electron acceptors for metal-reducing bacteria. Hery et al. [47] has proposed that Fe (III) reduction in aquifer sediments could play a role in as release through co-reduction of arsenate under appropriate conditions. Referring to the experimental results, the as and Fe released was closely linked to the availability of As-bearing Fe minerals in the presence of RB enrichments (Figure 2). While the contents of solid iron phase were comparable between sediments at two depth intervals (ca. 34,000 mg kg^{-1}), contents of solid-phase As were higher in 18-m core sediment than in 16-m (9.0 mg kg^{-1} at 16 m versus 12.1 mg kg^{-1} at 18 m). According to the experiment results, arsenic mobilized by microbial processes accounted for 5 and 12% of the solid As in 16- and 18-m sediments, respectively (Figure 2). Assuming the RB enrichment cultures using specific Fe mineral types in the sediment, the disparate as valence in end-stage sediments confirmed the limited availability of As-bearing Fe minerals (Figure 4).

Thus, the differing volumes of As released in the groundwater are affected by not only the microbial activities, but also the bioavailable amounts of organic carbon and As adsorbed onto or coprecipitated with Fe minerals in the sediment used. To graphically illustrate the results of the study, a conceptual model of microbial reductive processes of As

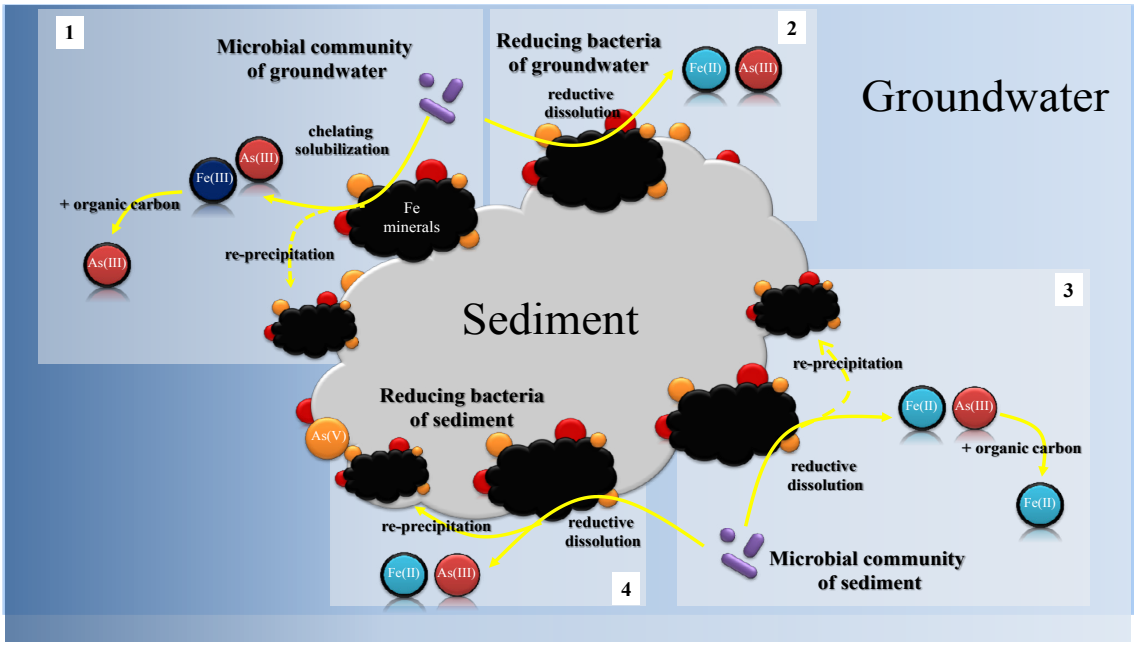

Figure 5: A conceptual diagram of different microbial processes involved in arsenic biochemistry in the As-contaminated aquifer. Four kinds of microbial population are considered, including (1) microbial community of groundwater, (2) reducing bacteria cultivated from groundwater, (3) microbial community of sediment, and (4) reducing bacteria cultivated from sediment. The solid and dash lines represent the major and minor mechanisms, respectively. The thick and thin circles represent the main and minor release components, respectively.

and Fe in the As-contaminated sedimentary aquifer is shown in Figure 5. The reducing bacteria in groundwater showed strong affinity for solid As, leading to enhance the high level of aqueous As. However, the reducing bacteria from sediment exhibited high reducing ability toward Fe-minerals, leading to sequestrate aqueous As by the formation of secondary Fe-As-minerals.

Conclusions

This study compared the medium-related microbial communities in liberating As and Fe in As-enriched aquifer. The microcosm experiments were systematically conducted to assess microbially mediated reactions for the mobility of As from sediment into groundwater. The distinct behaviors of As release were mainly governed by the contrasting microbial community cultured from groundwater (RB-W) and sediment (RB-S). For inoculated RB treatments, the reductive dissolution of Fe minerals resulted in preliminary mobilization of As, followed by the potential formation of secondary Fe minerals. By amending with different sediments, the bioavailability of Fe indeed affected the release amounts of As.

Addition of acetate as a degradable source of organic carbon significantly altered the sequence of terminal electron acceptors, reflecting on the decoupled processes of As and Fe under biotic condition, which was resuspended with non-sterile groundwater. Chelating solubilization of As-bearing Fe oxyhydroxides, which was relevant to the in-situ mobile phase, provided another potential pathway for As mobility. These results suggested that microorganism-based reactions, either chelating solubilization or reducing dissolution of Fe oxyhydroxides, were crucial for the formation of As-contaminated water in the subsurface. The bioavailability of labile carbon and As affected the quantity and distribution of aqueous As in the subsurface environment. These microcosm-based experiments can provide further understandings of microbial processes affecting sequestration and mobilization of As in arsenic contaminated aquifers.

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